Official Study Title: A Phase I/II Study Evaluating SJCAR19 (CD19-Specific CAR Engineered Autologous T-Cells) in Pediatric and Young Adult Patients ≤ 21 Years of Age With Relapsed or Refractory CD19+ Acute Lymphoblastic Leukemia

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SJCAR19: A PHASE I/II STUDY EVALUATING SJCAR19 (CD19-SPECIFIC CAR ENGINEERED AUTOLOGOUS T-CELLS) IN PEDIATRIC AND YOUNG ADULT PATIENTS ≤ 21 YEARS OF AGE WITH RELAPSED OR REFRACTORY CD19^{+} ACUTE LYMPHOBLASTIC LEUKEMIA

IND # 18164

Principal Investigators
Aimee C Talleur, MD[1]
Stephen Gottschalk, MD[1]

Co-Investigators

Brandon M. Triplett, MD[1]
Ewelina Mamcarz, MD[1]
Ashok Srinivasan, MD[1]
Renee Madden, MD[1]
Amr Qudeimat, MD[1]
Paulina Velasquez, MD[1]
Salem Akel, PhD[1]
Akshay Sharma, MD[1]
Cheng Cheng, PhD [2]
Swait Naik, MBBS[1]
Caitlin Zebley, MD[1]
Ali Suliman, MD[1]
Rebecca Epperly, MD[1]
Terrence Geiger, MD PhD[3]
Hiroto Inaba, MD, PhD[6]
Esther Obeng, MD, PhD[6]
Benjamin Youngblood, PhD[4]
Paul Thomas, PhD[4]
Charles Mullighan, MBBS, MD[3]
Allison Bragg, PharmD[5]
Justin Baker, MD[6]
Deena Levine, MD[6]
Liza Johnson, MD[6]
Jiyang Yu, PhD[7]
Senthil Bhoopalan, MBBS, PhD[1]

Department of Bone Marrow Transplantation and Cellular Therapy[1]
Department of Biostatistics[2]
Department of Pathology[3]
Department of Immunology[4]
Department of Pharmaceutical Services[5]
Department of Oncology[6]
Department of Computational Biology[7]

St. Jude Children's Research Hospital
262 Danny Thomas Place

Memphis, TN 38105
(901) 595-3300

TABLE OF CONTENTS

STUDY SUMMARY

Protocol Title

SJCAR19: A Phase I/II study evaluating SJCAR19 (CD19-specific CAR engineered autologous T-cells) in pediatric and young adult patients ≤ 21 years of age with relapsed or refractory $CD19^+$ acute lymphoblastic leukemia

Principal Investigators:
Aimee C Talleur, MD
Stephen Gottschalk, MD

Brief Overview

Study Population:

Patients less than or equal to 21 years old with relapsed or refractory $CD19^+$ acute lymphoblastic leukemia, as defined by protocol eligibility criteria. Additional eligibility criteria are specified to assure sufficient multi-organ system function.

Intervention, Brief Outline, and Objectives of Treatment Plan:

Modern frontline therapy for patients with $CD19^+$ acute lymphoblastic leukemia (ALL) is based on intensive administration of multiple drugs. In patients with relapsed or refractory disease response to chemotherapy is generally poor, and dosages cannot be further increased without unacceptable toxicities. For most patients, particularly those who relapse while still receiving frontline therapy, the only therapeutic option is hematopoietic cell transplantation (HCT). However, HCT is an intensive therapeutic modality with a significant risk of morbidity and mortality, and has limited efficacy in patients unable to achieve remission prior to HCT. Improvement in cure rates requires the development of treatments that bypass cellular mechanisms of drug resistance and have high therapeutic indexes. One such treatment is chimeric antigen receptor (CAR) engineered T-cells, a form of adoptive immunotherapy that has shown immense promise for treating relapsed or refractory $CD19^+$ ALL.

SJCAR19 is a Phase I/II clinical trial evaluating the use of SJCAR19 (CD19-specific CAR engineered autologous T-cells) in patients ≤ 21 years old with relapsed/refractory $CD19^+$ ALL. The study will contain a three part eligibility criteria: one for autologous apheresis, a second to proceed with manufacturing of the SJCAR19 product and a third to proceed with SJCAR19 therapy. A single patient cohort will be evaluated, without modifications to therapy based on either disease burden or prior allogeneic HCT status. Treatment will include a single treatment course, with most patients receiving a lymphodepleting chemotherapy preparative regimen of fludarabine/cyclophosphamide, followed by a single infusion of SJCAR19 (with dosing based on the number of CAR^+ T cells and patient weight). The evaluation period for the primary objectives of both the Phase I and II portions of the study will be 4 weeks. Long-term follow-up will occur for 15 years as per FDA guidance. Follow-up will occur on protocol for the 1 year after the last SJCAR19 infusion. After one year, patients will be approached and consented to our existing long-term follow-up (LTFU) institutional protocol. If a patient does not enroll on this LTFU protocol, we will continue to monitor patients on-study yearly, for up to 15 years post-infusion, per FDA guidance.

The Phase I portion will evaluate the safety and maximum tolerated dose (MTD) of SJCAR19, using a standard 3+3 study design and a 4 week post infusion of SJCAR19 evaluation period.

The Phase II portion will evaluate the efficacy, and provide further safety evaluation, of SJCAR19 in an expansion cohort at the MTD determined in the Phase I portion of the study. Patients treated at the MTD in the Phase I portion of the study will be evaluated for efficacy in the Phase II portion. Patients enrolled on the Phase II study and who meet protocol defined criteria, may receive subsequent therapy with lymphodepleting chemotherapy and reinfusion of the SJCAR19 product.

IND/IDE: IND #18164

Criteria for Evaluation – Safety and Efficacy
Safety: Ongoing assessment of toxicity will be done using the NCI CTCAE version 5.0. Cytokine release syndrome and neurotoxicity will be graded and assessed based on ASTCT consensus grading system similar to that used in established CAR^+ T-cell clinical trials (appendix G and H). The evaluation period for dose-limiting toxicities will be 4 weeks post-infusion of SJCAR19.
Efficacy: The primary measure of efficacy will be the complete response rate at 4 weeks post-infusion of SJCAR19.

Statistical Considerations and Data Analysis
Study Design: Phase I/II
Randomization: No
Sample Size: Total: up to 24 evaluable patients; Phase I: 9-12 patients, Phase II: 12 patients
Data Analyses:
Anticipated primary completion date: July 1, 2023
Anticipated study completion date: July 1, 2024
Time frame for primary outcome measure: 4 weeks post-SJCAR19 infusion

Data Management including statistical evaluations
Protocol compliance, data collection including safety data, and reporting will be carried out by the Department of Bone Marrow Transplantation and Cellular Therapy Research Office.
Statistical considerations and ongoing analysis will be conducted by Dr. Cheng Cheng and designated associates within the St. Jude Department of Biostatistics.

Human Subjects:
The risks to participants are primarily related to autologous apheresis, the conditioning regimen, the cellular infusion and *in vivo* toxicity of SJCAR19. CD19-specific CAR T-cell therapy has been associated with cytokine release syndrome, neurotoxicity and B-cell aplasia. Adverse events will be treated, monitored, and reported appropriately. Possible benefits of participation include obtaining and/or sustaining disease remission. In addition, there is the possibility of psychological benefit from knowing participation has helped researchers gain more understanding about SJCAR19. Potential alternatives to participation include chemotherapy, HCT, other research treatment if available, and/or supportive therapy alone. The possible benefits, alternatives to participation, and side effects, including that there may be unknown side effects of treatment, are detailed in lay language within the respective informed consent document.

1.0 OBJECTIVES

1.1 Primary Objective

1.1.1 The primary objectives for the Phase I study portion are to determine the maximum tolerated dose (MTD) and characterize the safety profile and dose-limiting toxicities (DLTs) of treatment with SJCAR19 in pediatric and young adult patients ≤ 21 years of age, with relapsed or refractory $CD19^+$ ALL.

1.1.2 The primary objective for the Phase II study portion is to evaluate the complete response (CR) rates of SJCAR19 in pediatric and young adult patients ≤ 21 years of age, with relapsed or refractory $CD19^+$ ALL.

1.2 Exploratory Objectives

1.2.1 To describe the feasibility of manufacturing SJCAR19 for pediatric and young adult patients ≤ 21 years of age, with relapsed or refractory $CD19^+$ ALL, and explore possible factors contributing to manufacturing failure.

1.2.2 To evaluate the relapse-free survival of patients with minimal residual disease at the time of treatment with SJCAR19.

1.2.3 To study the expansion, persistence and phenotype of SJCAR19.

1.2.4 To characterize the cytokine profile in the peripheral blood and CSF after treatment with SJCAR19.

1.2.5 To explore the use of next-generation sequencing (NGS) for the monitoring of disease status post-treatment with SJCAR19 compared to minimal residual disease detection via flow cytometry.

1.2.6 To assess whether SJCAR19 cells acquire functional versus exhaustion-associated epigenetic programs during in vitro and in vivo expansion.

1.2.7 To determine immune reconstitution post SJCAR19 treatment, and the clonal structure and endogenous repertoire of SJCAR19 cells during in vitro and in vivo expansion.

1.2.8 To longitudinally assess and quantify the symptoms, associated distress, and functional impairment experienced by patients enrolled on this Phase I/II clinical trial.

1.2.9 To longitudinally assess and quantify numerous metrics of quality of life and well-being for patients enrolled on this Phase I/II trial and their primary caretakers.

1.2.10 To characterize incidence and mechanisms of resistance and/or relapse post-therapy with SJCAR19.

2.0 BACKGROUND AND RATIONALE

2.1 Overview

SJCAR19 is a Phase I/II clinical trial evaluating the use of SJCAR19 (CD19-specific CAR engineered autologous T-cells) in pediatric and young adult patients ≤ 21 years old, with relapsed/refractory $CD19^+$ acute lymphoblastic leukemia. This study will evaluate the safety and maximum tolerated dose (Phase I), and efficacy (Phase II) of SJCAR19. The study will contain a three part eligibility criteria: one for autologous apheresis, a second to proceed with manufacturing of the SJCAR19 product and a third to proceed with SJCAR19 therapy. Treatment will include a single treatment course, with most patients receiving a lymphodepleting chemotherapy preparative regimen of fludarabine/cyclophosphamide, followed by a single infusion of SJCAR19 (with dosing based on the number of CAR^+ T cells and patient weight). Patients enrolled on the Phase II study and who meet protocol defined criteria, may receive subsequent therapy with lymphodepleting chemotherapy and reinfusion of the SJCAR19 product. Primary objectives will be evaluated at 4 weeks post-infusion with SJCAR19. Long-term follow-up will occur for 15 years as per FDA guidance. Follow-up will occur on protocol for the 1 year after the last SJCAR19 infusion. After one year, patients will be approached and consented to our existing long-term follow-up (LTFU) institutional protocol. If a patient does not enroll on this LTFU protocol, we will continue to monitor patients on-study yearly, for up to 15 years post-infusion, per FDA guidance.

2.2 Relapsed and Refractory Acute Lymphoblastic Leukemia

Acute lymphoblastic leukemia (ALL) accounts for approximately 25% of new pediatric cancer cases diagnosed each year in the United States. Although cure rates for pediatric ALL have steadily improved and are now nearing 90%, patients with relapsed or refractory disease do much poorer.[2, 3] Treatment options are typically limited for these patients, and therapy relies primarily on hematopoietic cell transplantation (HCT).[4-7] Second remissions are achieved in the majority of relapsed ALL patients but overall cure rates seldom exceed 30%, with leukemic relapse after HCT remaining the most common cause of failure.[8-12] Relapse after HCT can be even more difficult to treat due to the development of increased resistance to chemotherapy, resistance to donor lymphocyte infusions, and often multiple comorbid conditions secondary to disease or prior treatments, all of which can limit therapeutic choices.[13] Significant improvement in cure rates requires the development of treatments that bypass cellular mechanisms of drug resistance and have high therapeutic indexes.

2.3 Chimeric Antigen Receptors

Chimeric antigen receptors (CARs) have been developed as a form of adoptive immunotherapy. CARs typically combine the single chain variable fragment (scFv) of an antibody or the extracellular domain of a receptor, with a transmembrane domain

and the intracellular signaling domains of the CD3ζ chain and costimulatory molecules. A CAR can be constitutively expressed on the surface of a T-cell through non-viral or viral transduction, thereby enabling a cytotoxic T-cell to recognize certain targets in an antigen-specific manner.[14-17] CARs can be designed to target a specific tumor-associated antigen and thereby can be used for anticancer therapy.[18-20] Different generations of CARs have been developed in attempts to improve persistence and expansion and efficacy, including the addition of costimulatory receptors such as CD28 and 4-1BB.[16, 17, 21-25]

2.4 Rationale for CD19-specific Chimeric Antigen Receptor Therapy

Leukemia cells can be delineated by the surface markers they express, and these markers are potential targets for immunotherapy. CD19 is one such surface marker. CD19 is a 95kDa glycoprotein. It is found on the surface of B cells from early on in development until they differentiate into plasma cells, but is not found on hematopoietic progenitor cells.[26-29] CD19 is an attractive target for CAR therapy because its expression is restricted in normal host tissues to B lineage cells, but is also expressed by most B cell ALL cells.[25, 28]

Several pediatric institutions and pharmaceutical companies are currently investigating the use of autologous CD19-specific CAR T-cell therapy in a relapsed/refractory $CD19^+$ ALL disease patient cohort, and have seen great success to date.[16, 19, 21, 30-32] Major differences between these ongoing trials include vector construct and costimulatory endodomain (CD28 vs 4-1BB), lymphodepleting chemotherapy regimens prior to the CAR T-cell infusion, CAR T-cell doses/schedules and disease specific inclusion criteria. Despite these differences, the efficacy and toxicities, as outlined below, have been similar across various institutions.

Seattle Children's Hospital: Investigators are evaluating a lentiviral vector that encodes a CD19-specific CAR with a 4-1BB.ζ endodomain and truncated EGFR separated by a 2A sequence. The use of pre-lymphodepleting chemotherapy is optional and initial cohorts are determined based on prior allogeneic transplant status. Infusion doses are based on the number of CAR^+ T cells and the product is composed of defined CD4:CD8 T-cell subsets. Preliminary results from their Phase I trial including 13 patients, shows a molecular remission rate of 85%.[33]

National Institute of Cancer: Investigators have evaluated a retroviral vector that encodes a CD19-specific CAR with a CD28.ζ endodomain. Treatment cohorts are determined by level of disease burden. The lymphodepleting chemotherapy regimen varies based on cohort, and dosing levels use the number of CAR^+ T cells. Intent to treat analysis of 21 patients demonstrated a complete response rate of 67%.[33, 34] This CD19-specific CAR T-cell product (KTE-C19) is now being evaluated by KITE Pharma in a Phase II multicenter trial. All patients receive a lymphodepleting chemotherapy regimen of fludarabine and cyclophosphamide, and KTE-C19 dosing is determined by the number of CAR^+ T cells and patient weight. Initial reports show

that the regimen appears to be safe and feasible, with promising efficacy, but numbers are small at this time.[35]

Memorial Sloan Kettering: Investigators are evaluating a retroviral vector that encodes a CD19-specific CAR with a CD28.ζ endodomain in two cohorts that are defined by level of disease burden. The conditioning regimen is uniform for all participants and dosing is based on the number of CAR^+ T cells. This study does not yet have reportable results, but a similar study design in adult patients demonstrated a 28-day complete response rate of 88%.[33]

Children's Hospital of Philadelphia/University of Pennsylvania: Investigators have evaluated a lentiviral vector that encodes a CD19-specific CAR with a 4-1BB.ζ endodomain (CTL019), using varied lymphodepleting regimens. Dosing levels are determined by the total number of T cells, not CAR^+ T cells. The reported 12-month overall survival rate for 53 patients is 78%.[22, 32] CTL019 was also evaluated in a multi-center trial (ELIANA) by Novartis. Analysis of this trial showed a 12-month overall survival rate of 76% and event-free survival rate of 50%.[36, 37] In 2017, this product (Kymriah) received FDA approval for the treatment of patients aged 1 – 25 years old with refractory or relapsed (2^{nd} or greater) CD19+ ALL.

2.5 Rationale for Lymphodepleting Preparative Regimen

The lymphodepletion of host T cells prior to the adoptive transfer of CAR T-cells may enhance the effectiveness and survival of the CAR T-cells. T cell homeostasis is controlled by several factors, including access to antigen-presenting cells (APCs), major histocompatibility complex (MHC) presentation of self and foreign peptides and antigens, interactions with regulatory T cells, and competition for cytokines.[38, 39] The elimination of host lymphocytes allows transferred cells increased access to APCs and peptides being presented by MHC. It also eliminates regulatory T-cells, and other competing elements of the immune system that act as cytokine sinks, allowing for increased availability of cytokines to be used by transferred T cells.[39-41]

The chemotherapy agents fludarabine and cyclophosphamide are commonly used for lymphodepletion.[38] This drug combination has been used in several CD19-specific CAR T-cell therapy trials.[22, 34, 35, 42, 43] Studies have shown that the addition of fludarabine to cyclophosphamide improves the persistence and expansion of CAR-engineered T cells[44], in part possibly due to the effects of the chemotherapy agents on host cytokines and chemokines.[45]

2.6 Safety issues with CD19-specific CAR T-cell Therapy

Potential adverse events from CD19-specific CAR T-cell therapy include those related to 1) the apheresis of autologous cells, 2) the risks of the preparative regimen agents, 3) the infusion of the cellular product, 4) general consequences of lentiviral transduction and 5) *in vivo* consequences related to therapy with CD19-specific CAR T-cells.

2.6.1 Apheresis of Autologous Cells

Autologous apheresis is performed to obtain the peripheral blood mononuclear cells needed for manufacturing CAR T-cells. Apheresis is performed using either a peripheral or temporary central line. During the collection, donors are exposed to drugs to prevent their blood from clotting; these drugs may include citrate and/or heparin. Donors are monitored closely for side effects from these drugs, including hypocalcemia and bleeding. After donation, donors may experience a decrease in platelet or red blood cell counts, requiring the need for transfusion. Overall, apheresis is generally well tolerated.

2.6.2 Lymphodepleting Chemotherapy

The chemotherapy agents fludarabine and cyclophosphamide are commonly used as a lymphodepleting preparative regimen.[38] This drug combination has been used in several CD19-specific CAR T-cell therapy trials.[22, 34, 35, 42, 43] Both drugs are commercially available and a list of their toxicities are included in Section 5.1.

2.6.3 Infusion of CAR product

CAR-engineered T cell products contain a percentage of transduced and untransduced T cells. All of the cells have undergone *ex-vivo* activation and expansion. Studies have evaluated the risks of infusion of *ex-vivo* activated autologous T cells, with minimal side effects noted.[46, 47] Additionally, CD19-specific CAR autologous T cell trials have not reported significant infusion-related reactions.[22, 34, 48]

2.6.4 General Risks of Lentiviral Transduction

Efficient and stable expression of CD19-specific CARs in T cells requires the use of viral vectors for delivery. The use of viral vectors has a potential risk of cell transformation resulting from insertional mutagenesis,[49] a risk that appears to be dependent on the therapeutic gene carried by the vector,[50] and/or on the cell population targeted by the vector.[51] Lentiviruses are a subclass of the retrovirus family. Lentiviral vectors are commonly used in CD19-specific CAR T-cell therapy trials.[22, 35, 36 33] Lentiviral vectors are capable of stably integrating into a target cell's genome, including quiescent cells. Several strategies have been employed to reduce the risk of insertional mutagenesis, including the use of self-inactivating (SIN) vectors, chromosomal insulators and tissue-specific promotors.[51, 52] Additionally, a risk of using lentiviral vectors is the generation of a replication competent lentivirus (RCL) during the manufacturing process. This risk is minimized by current vector manufacturing practices.[53] Long term follow-up of human trials using lentiviral vectors have thus far not reported oncogenic mutagenesis.[54, 55]

2.6.5 Post-Infusion Risks

Based on other studies of CD19-specific CAR T-cell therapy, common toxicities include cytokine release syndrome, neurotoxicity and B-cell aplasia.[25, 31, 48, 56-58]

2.6.5.1 Cytokine Release Syndrome

Cytokine release is induced by activated CAR T-cells after engagement with their target. This cytokine release causes varying degrees of a systemic inflammatory response, referred to as cytokine release syndrome (CRS). CRS typically occurs within the first 1-2 weeks after infusion of the CD19-CAR T-cell product, correlating with the timing of maximal CAR-T expansion and proliferation.[56, 59] The most commonly observed manifestations have included fever, hypotension, vascular leak, renal complications with a rise in creatinine or renal failure, and pulmonary complications with hypoxia or pleural effusions. CRS can generally be medically managed with supportive care; in severe cases, CRS has been treated with monoclonal antibody therapy against IL-6 or TNF-alpha, and corticosteroids.[56-58, 60-62]

2.6.5.2 Neurotoxicity

Neurologic changes after receipt of CD19-CAR T-cells have been reported. Similar to CRS, these events often correlate with peak T-cell expansion and cytokine release, but can also occur independently of CRS. The spectrum of observed neurologic toxicities is vast, without localization to any one neuroanatomic location. Reported toxicities include headache, confusion, hallucinations, altered mental status, encephalopathy, ataxia, cranial nerve palsy, seizures, apraxia, dysphagia and tremors.[57, 58] No clear patient or treatment factors have been elucidated that can predict the development of neurotoxicity, including the presence of CNS leukemia.[56] Observed neurotoxicities have generally been brief and self-limited, resolving over several days and without long term deficits.[25] Providing prophylactic antiepileptic medication is not standard of care, but may be considered on a case by case basis.

2.6.5.3 B-cell Aplasia

Because CD19 is universally expressed on B-cells, including early B-cell precursors, recipient B-cell aplasia is an expected on-target/off-tumor effect of CD19-specific CAR T-cell therapy. B-cell lymphopenia and hypogammaglobinemia are expected while CD19-specific CAR T-cells persist in the recipient, and should resolve if/when the CD19-specific CAR T-cells were to diminish.[33]

2.6.5.4 Risk of developing a “false positive” HIV test

After taking part in this study, participants may test positive for HIV on screening tests. There is a difference between testing positive on the screening test and actually having an HIV infection. There are no definite tests that can tell the difference between these two cases at the moment, but we will notify participants if a test is developed in the future.[63]

2.7 Rationale for Present Study

2.7.1 SJCAR19

Researchers at St. Jude have developed a second generation lentiviral CD19-specific CAR construct, containing a 4-1BB.ζ domain. This vector is being produced in the St. Jude GMP facility using a stable producer cell line.[64] Pre-clinical, translational evaluation of this CAR vector has proved promising (unpublished data, Figure 1), including in the allogeneic effector T-cell setting.[65] This is the first clinical study evaluating the safety and efficacy of this CAR construct for the treatment of pediatric relapsed or refractory $CD19^+$ ALL, with the final CAR-engineered autologous T-cell product being referred to as SJCAR19.

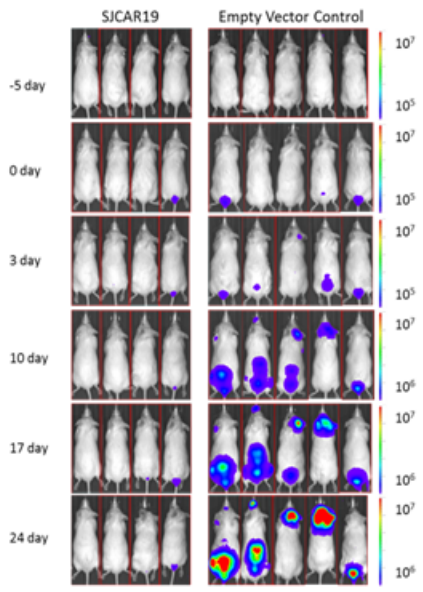


Figure 1: *In vivo* activity of SJCAR19 in NOD/scid/$\gamma C^{-/-}$ (NSG) mice model. NSG mice were injected intravenously (iv) with 1 x 10^6 Raji cells (B-cell lymphoma) expressing firefly luciferase. Seven days later (Day 0), SJCAR19 (4 x 10^5 cells) or empty vector control T-cells were administered iv. Serial bioluminescence imaging was used to track tumor cells *in vivo*.

2.7.2 Relapsed/Refractory ALL Criteria

Relapsed and refractory ALL is often difficult to treat due to increased chemoresistance of the leukemic blasts. Those with high-risk relapse, such as $CD19^+$ ALL with early relapse or history of slow response to up-front induction therapy, are typically referred for treatment with HCT as their chance of long-term cure with conventional therapy alone is dismal.[4] For those patients that relapse after initial HCT, or those that are unable to proceed to HCT for reasons such as disease status, donor availability or patient health status, treatment options are limited. Additionally, patients with refractory $CD19^+$ ALL are a high-risk, difficult to treat population.

As the overall success of HCT for ALL is largely dependent on the ability to achieve disease remission prior to HCT, having refractory disease significantly decreases the effectiveness of HCT.[66] Since these patients are chemorefractory, there is a need for therapeutic options that utilize non-chemotherapy treatment options that serve as a bridge to HCT, or an alternative to HCT.

The relapsed and refractory disease inclusion criteria for SJCAR19 are modeled after the disease classification criteria that is currently being used by the St. Jude Leukemia Division. They are intended to provide those patients with chemorefractory disease, either primary refractory or at time of relapse, a novel therapeutic option that may serve as a bridge to HCT or as a stand-alone treatment option. This includes those patients that may require HCT, but are unable to receive HCT due to ineligibility and/or unsuitability. Additionally, SJCAR19 will provide a treatment option for those patients that relapse after HCT or experience multiple relapses, a patient population that is historically difficult to treat using conventional therapy.

2.7.3 Early Autologous Apheresis

When manufacturing a CAR T-cell product the quality of the apheresis sample, specifically the quantity and/or quality of circulating T cells, can affect the ability to generate a final CAR-T product. However, it is not known when the "best" time to collect autologous cells is.[43, 67] Many CAR T-cell trials collect patient cells at a time in their disease process where they have already undergone several treatment modalities, and institutions have reported inabilities to manufacture adequate products for a small subset of patients.[33, 68] Therefore, in this study we are using a 3-part eligibility criteria: one for apheresis, one for manufacturing and one for treatment with SJCAR19. The goal of this design is to allow for the apheresis of some patients earlier in their disease process, prior to receiving months of treatment, in an attempt to maximize the ability to generate the SJCAR19 treatment product if/when it is needed. In addition to those patients that we anticipate will go on to be evaluated for SJCAR19 therapy (those considered to have refractory or relapsed disease), we are proposing apheresis of patients with $CD19^+$ ALL who have other high-risk features, such MRD $\geq$ 1% at the end of up-front induction therapy or Hypodiploid disease, independent of risk classification at that time. With this earlier apheresis, there is a possibility that some patients will undergo the apheresis procedure, but not ultimately be eligible for treatment with SJCAR19. Looking at data from Total XVI, St. Jude's most recent up-front leukemia trial, there were 6 patients classified as either low or standard risk $CD19^+$ ALL (1.2% of all $CD19^+$ ALL patients enrolled on study) that would have met our proposed MRD criteria for apheresis. All of these patients were eventually upstaged and classified as high-risk, and therefore would ultimately have met disease

criteria for therapy with SJCAR19. Therefore, we anticipate that the number of patients who undergo apheresis and do not end up meeting disease eligibility criteria for treatment with SJCAR19 to be minimal.

2.7.4 Dose Escalation

The dose escalation schema of SJCAR19 is based on the experiences and results of similar CD19-specific CAR T-cell therapy protocols in pediatric and young adult patients with relapsed/refractory $CD19^+$ ALL. The Seattle Children's Phase I trial has infused a range of $5 \times 10^5 - 5 \times 10^6$ CAR^+ T-cells/kg. A MTD has not yet been reported.[33] The Phase I study at the National Cancer Institute determined the MTD of their retroviral product to be 1×10^6 CAR^+ T-cells/kg.[33, 34] Memorial Sloan Kettering is infusing either 1×10^6 or 3×10^6 CAR^+ T-cells/kg, depending on patient disease status at time of enrollment. The group at Children's Hospital of Philadelphia has infused a wide range of CAR+ T-cells, ranging from $1 - 17.36 \times 10^6$ CAR^+ T-cells/kg.[22] The ELIANA trial administered $2 - 5 \times 10^6$ CAR^+ T-cells /kg, with a maximum dose of 2×10^8 CAR^+ T cells for those patients weighing $\geq$ 50kg.[36] ZUMA-4 is evaluating $1 - 2 \times 10^6$ CAR^+ T-cells/kg, and have not yet reported a patient experiencing a dose limiting toxicity.[35] Additionally, the recently FDA approved CD19-specific CAR T product, Kymriah, provides dosing ranges that are based on the experience in these trials. Therefore, from this collective data, we are proposing a starting dose of SJCAR19 of 1×10^6 CAR^+ T cells/kg, and will include a standard maximum dose.

2.7.5 SJCAR19 Biology: Epigenetics and T-cell Exhaustion

While CAR T-cells undergo robust expansion upon transfer into patients containing antigen-presenting tumor cells, several studies have indicated that they subsequently undergo functional exhaustion during their anti-tumor response.[69, 70] This functional impairment often results in significant contraction in the total number of circulating CAR T-cells, leaving the host without long-lived immunity to the tumor. Several studies have demonstrated that CD8 T-cell exhaustion manifests through changes in gene regulation that can be reinforced and heritably maintained.[71-73] Transcriptional memory is often mediated by epigenetic modification to the genome, therefore we will use exhaustion-specific epigenetic programs as biomarkers to delineate between nonfunctional and functional CAR T-cells during product development and following adoptive transfer.

The following work is ongoing in the Youngblood laboratory at St. Jude. They have recently determined that conditional deletion of the DNA methyltransferase 3a (Dnmt3a cKO) inhibits acquisition of de novo DNA methylation programs in antigen-specific CD8 T-cells and prevents T-cell exhaustion during chronic viral infection. Importantly, Dnmt3a deficient antigen-specific CD8 T-cells retain their ability to mount a polyclonal effector response, and ultimately control the chronic pathogen. Moreover,

Dnmt3a cKO cells remain resistant to T-cell exhaustion even when the cKO cells are forced to persist in an environment that has artificially high levels of antigen for several months. Published and unpublished data serve as proof of principle that acquired epigenetic programs promote T-cell exhaustion, and can be used as a biomarker to assess the developmental state of the cell. They have recently developed protocols for performing whole-genome methylation analysis using low input genomic DNA, and have applied this methodology to determine the epigenetic signature of functional human memory CD8 T-cells (Figure 2). From their whole-genome analysis they have identified differentially methylated regions among the memory T cell subsets and have developed a PCR based assay to define the developmental status of the T cells.[1] These data provide insight into the poised state for expression of critical effector molecules and inhibitory receptors among the memory CD8 T-cell subsets.

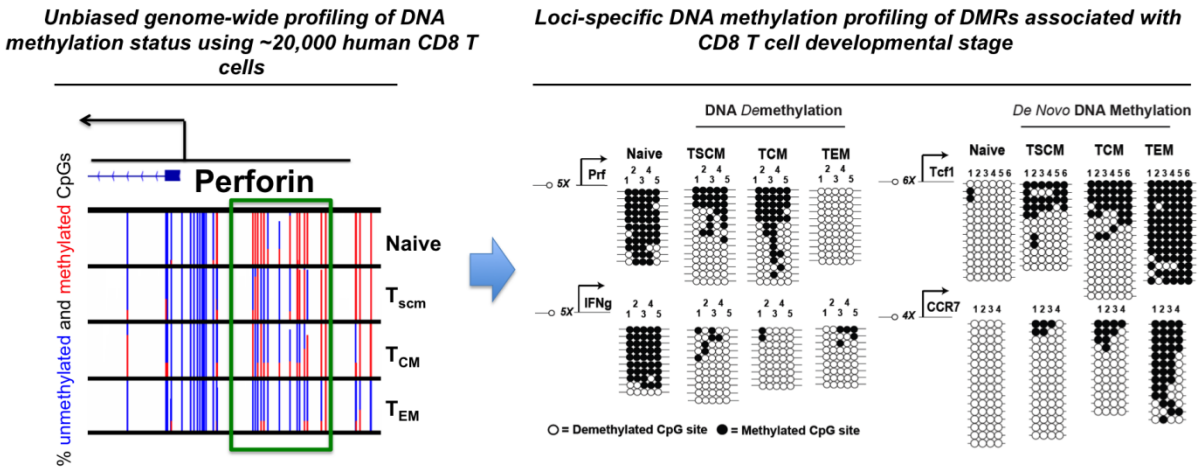


Figure 2: Whole-genome bisulfite sequencing of human T-cell subsets identifies demethylated and de novo methylated loci. WGBS methylation profiling provides nucleotide resolution methylation profiling maps of rare T-cell populations and can be used to identify differentially methylated regions (DMRs) that are coupled to function. A representative map of WGBS profiling of healthy naïve and functional memory T-cell subsets (Tscm, Tcm, Tem) is shown for the perforin locus. Red and blue lines represent methylation locations. This assay has identified DMRs in effector and lymphoid homing loci that correlate with the known cell subset-specific phenotypic and functional properties. Demethylation and de novo methylation DMRs will be validated with loci-specific PCR assays. Representative demethylation and de novo DNA methylation DMRs are shown for perforin, interferon gamma, Tcf1, and CCR7 promoters. WGBS and loci-specific data were collected at the St Jude Hartwell Sequencing Facility.[1]

In this study, we will perform methylation analyses of CAR T-cells pre infusion and following adoptive transfer, to measure changes in epigenetic programs that are coupled with the stable changes in the developmental status of the cells. Loci-specific analyses can be performed with a few hundred cells, and they have currently optimized conditions to perform whole-genome bisulfite sequencing on ~20,000 cells. Loci-specific analyses may include regions of the genome that code for effector

molecule, inhibitory receptor, and homing molecule loci including, granzyme b, perforin, PD-1, CTLA4 Lag3, 2B4, and CD62L. They will perform loci-specific analyses with samples that yield <10,000 PD-1+ T-cells. Whole genome bisulfite sequencing may be performed on samples that yield >10,000 PD-1+ T-cells.

2.7.6 SJCAR19 Biology: Clonal Structure and Endogenous Repertoire

CAR T-cells each contain an identical antigen receptor that mediates their anti-tumor function. However, these transduced receptors do not replace the endogenous receptors present in these T-cell populations prior to transduction with the CAR construct. Investigators in the Thomas laboratory at St. Jude, and others, have demonstrated in mouse and human T-cells that the T cell receptor can serve as a unique "barcode" for a clonal lineage, as each receptor within an individual is generally found only in a single naïve precursor T-cell.[74] Thus, by using techniques to sequence the T-cell receptor repertoire in a quantitative manner in combination with the HLA type of the CAR-T cell recipient, either by single cell RT-PCR approaches, or UMI-RT-PCR bulk methods, we can determine whether individual clonal lineages preferentially expand *in vitro* and *in vivo*.[75] Despite containing an identical receptor, stochastic effects and cross-talk from the endogenous receptor may cause differential expansion of individual clonal lineages, as has been observed for transgenic T-cells in mice.[76] Excessive expansion of only a few "founder" clones could contribute to accelerated exhaustion. Thus, this analysis will address two questions relevant for CAR T-cell therapy: 1) Does uneven expansion of founder populations occur in CAR T-cell therapy? If so, 2) are there correlations between the specificity of endogenous antigen receptors and preferentially expanded clones?

This study will include repertoire analysis (including use of HLA) and quantitative assessment of repertoire diversity and specificity will be used on longitudinal samples from *in vitro* and *in vivo* expanded CAR T-cell populations.[77] Outcomes will include the diversity (assessing both richness and evenness) of CAR T-cell and unmodified immune cell populations over time and the identification of receptor families from known specificities (e.g. common infections such as influenza, herpesvirus etc) that preferentially associate with expanded clones. Functionality of immune responses may be explored using standard methods including Elispot assays or determining, for example, the viral load by PCR analysis. Additionally, lentiviral vector integration site (VIS) analyses will be performed to track the clonal structure of genetically-modified CAR T cells.

2.7.7 Patient Reported Symptoms and Quality of Life

It has previously been shown that clinician-reported adverse event data underestimates the number and severity of symptoms patients are

experiencing.[78] However, patient reported outcomes have been shown to be more reliable measures of adverse events in the adult population. Patient symptoms can be prospectively assessed using the Patient Reported Outcomes version of the Common Terminology for Adverse Events (PRO-CTCAE). The PRO-CTCAE has been validated in adults, and inclusion of the adult PRO-CTCAE is now expected in any National Cancer Institute-funded clinical protocol. There is increasing interest in using patient reported outcomes to assess symptoms in pediatric oncology patients,[79, 80] and the PRO-CTCAE is currently undergoing validation studies at multiple pediatric cancer centers, including at St. Jude. We expect that the PRO-CTCAE will be fully validated in the pediatric population by completion of the SJCAR19 protocol. By including this novel tool to prospectively assess patient symptoms over time, we expect that SJCAR19 will be one of the first clinical trials to report adverse event data in pediatric patients using the PRO-CTCAE.

Patient and caretaker quality of life may be affected by physical symptoms, medical treatments, social support, ability to participate in activities and social functioning. Evaluation of the quality of life (QoL) of research participants and their primary caretakers has been incorporated into several clinical trials at St. Jude. As part of this current study, we plan to assess patient and primary caretaker quality of life and symptomatology using a similar battery of instruments to those used on the previous clinical trials SJPDGF and SJHG12. These evaluations consist of questionnaires to be filled out by research participants and primary caretakers, as well as semi-structured interviews. In this study, we also intend to use semi-structured interviews to qualitatively probe patient symptomatology in hopes of gaining a better understanding of the experience of poorly characterized CAR T-cell-specific side effects, including neurologic changes.

Little is known about the effect of caretaker activation on measures of caretaker quality of life, especially during an intense treatment phase. Research has demonstrated that adults with greater activation have better healthcare experiences, and that interventions aimed at increasing activation are associated with improved health and healthcare quality.[81] In this study we will assess primary caregiver activation, defined as the primary caregivers' willingness to manage the patient's health and healthcare and is based on the caretaker's knowledge, skills, and confidence in managing the patient's health. We will also longitudinally assess numerous aspects of primary care giver quality of life including hope, anxiety and depression, and spiritual well-being. We hope that exploring the relationship between QoL and activation to inform further research in this developing field of study.

When possible, questionnaires will be administered in the participant's native language. All questionnaires are available in English. A subset of questionnaires have been validated in Spanish. We will use these validated Spanish questionnaires for Spanish-speaking participants. Semi-structured interviews will only be conducted in English as the logistics of using interpreter services for this purpose are challenging and the validity of interpreted qualitative data is uncertain. Non-English-speaking participants will not participate in the qualitative symptom assessment or the good day/bad day scale.

2.7.8 Exploration of resistance/relapse mechanisms

The appearance of leukemia clones resistant or refractory to adoptive immunotherapy have been described in clinical trials treating patients with ALL with CD19- and CD22-CAR T-cells. Identified resistance mechanisms are varied, including antigen downregulation, alternative splicing and lineage switch.[88-92] To further investigate mechanisms of relapse, we plan to perform analysis on leukemic blast samples as well as on the tumor microenvironment. This will include flow cytometric analysis to investigate antigen downregulation and overexpression of inhibitory ligands, analysis of HLA/haplotypes, as well genomic, transcriptomic and proteomic analysis of the relapsed leukemia populations.

2.7.9 Reinfusion of CD19-CAR T-cells (Amendment 5.0)

While treatment with CD19-CAR T-cells has shown to induce remission in the majority of pediatric ALL patients, duration of efficacy is variable. Given the multiply relapsed and/or refractory population that is typically treated with CD19-CAR T-cell therapy, treatment options for recurrent leukemia after CAR-T are extremely limited. Therefore, a primary goal of CAR T-cell therapy is to provide long-term remission.

One mechanism of relapse is the loss of functional CAR T-cells, possibly due to poor T-cell fitness/exhaustion or immune mediated rejection. A marker of diminishing CD19-CAR T-cell activity is autologous B-cell recovery. The timing of which has been shown to associate with risk of relapse, such that patients who experience early recovery (often defined as $\leq$ 6 months after infusion) have a higher relapse risk. One strategy to decrease relapse risk and maintain longer term efficacy is to monitor closely for B-cell recovery and, with evidence of early recovery, provide repeated infusions of the CAR T-cell product. While early B-cell recovery can be a sign of impending relapse, some patients experience both nearly concurrently and if disease remains CD19+, repeat infusion of the initial CAR T-cell product may again provide efficacy. Given the concern for rejection as a mechanism for loss of CAR T-cells, incorporation of an intensified lymphodepleting regimen has often been employed to reduce the likelihood of immune rejection of the reinfused CAR-T cells.

In our experience with patients treated on the Phase I portion of SJCAR19, 4/12 patients were evaluable for early recovery of autologous B-cells (evaluable defined as a responding patient with serial analysis of B-cells in the peripheral blood for ≥ 2 months post-SJCAR19 infusion). In these 4 patients, 3 had autologous B-cell recovery ≤ 6 months after initial infusion and all had subsequent recurrence of detectable CD19-positive disease. These 3 patients went on to receive a 2nd infusion of their SJCAR19 product on individualized single patient investigator plans (SPIPs), and 2/3 achieved a 2nd CR, with expected, minimal toxicities.

Therefore, in the Phase II portion of this study, we are including the option for reinfusion of the originally manufactured SJCAR19 product, for patients with evidence of early B-cell recovery (≤ 6 months after infusion) and/or detectable disease (≤ 12 months after infusion). This option will be reserved for patients who previously responded to their SJCAR19 infusion, have recovered from prior toxicities and continue to meet treatment eligibility criteria (section 3.3). We will include an intensified lymphodepletion regimen, with the option to use the 1st infusion lymphodepleting chemotherapy regimen (Table 1) per PI discretion. Dosing will not exceed the MTD.

2.7.10 Summary

SJCAR19 will be the first CAR T-cell clinical protocol at St. Jude Children's Research Hospital. We serve a relapsed/refractory CD19+ ALL patient population that is in need of new cancer-directed therapies. We are equipped to manufacture the SJCAR19 product in our Good Manufacturing Practice (GMP) facility, and have extensive clinical experience with high-risk patients and their associated complications. Other institutions evaluating CD19-specific CAR T-cell therapy have shown tremendous efficacy with tolerable toxicities, in this difficult to treat population. However, the field is generally new and there is still a tremendous amount of information to be learned, including related to the optimal timing of apheresis, processing schemas, management of side effects and CAR T-cell biology. At St. Jude, we will be able to further explore these questions and use this knowledge to contribute to the field, as well as to form the basis for our own developing CAR T-cell therapy program.

3.0 RESEARCH PARTICIPANT ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

The study will contain a three part eligibility criteria: one for autologous apheresis (section 3.1), a second to proceed with manufacturing of the SJCAR19 product (section 3.2) and a third to proceed with treatment with SJCAR19 (section 3.3).

According to institutional and NIH policy, this study will accession patients regardless of gender and ethnic background. Institutional experience confirms broad representation in this regard. However, pregnant and lactating females are excluded from participation as the short and long-term effects of the preparative agents and study infusions, as well as the long-term effects of the apheresis procedure on a fetus and a nursing child through breast milk are not entirely known at this time.

3.1 Eligibility Criteria for Autologous Apheresis

3.1.1 Inclusion Criteria for Autologous Apheresis

3.1.2.1 Age ≤ 21 years old

3.1.2.2 $CD19^+$ ALL with *any* of the following*:

- Minimal Residual Disease (MRD) ≥ 1% at end of up-front induction therapy
- Hypodiploid (< 44 chromosomes or < 0.95 DNA index) $CD19^+$ ALL with detectable disease at the end of up-front induction therapy
- Increase in disease burden any time after the completion of up-front induction therapy
- Primary refractory disease despite at least 2 cycles of an intensive chemotherapy regimen designed to induce remission
- Refractory disease despite salvage therapy
- 1st or greater relapse

*note: if patient met CD19+ ALL disease criteria, subsequent receipt of cancer directed therapy that eradicates disease does not preclude them from proceeding with this study

3.1.2.3 Estimated life expectancy of > 12 weeks

3.1.2.4 Karnofsky or Lansky (age-dependent) performance score ≥ 50 (Appendix A)

3.1.2.5 Patients with a history of prior allogeneic hematopoietic cell transplantation [HCT] must be clinically recovered from prior HCT therapy, have no evidence of active GVHD and have not received a donor lymphocyte infusion (DLI) within the 28 days prior to apheresis

3.1.2.6 For females of child bearing age:

- Not lactating with intent to breastfeed
- Not pregnant with negative serum pregnancy test within 7 days prior to enrollment

3.1.2 Exclusion Criteria for Autologous Apheresis

3.1.2.1 Known primary immunodeficiency

3.1.2.2 History of HIV infection

3.1.2.3 Severe intercurrent bacterial, viral or fungal infection

3.1.2.4 History of hypersensitivity reactions to murine protein-containing products

3.2 Eligibility Criteria for Manufacturing SJCAR19

3.2.1 $CD19^+$ ALL with *any* of the following*:

- Primary refractory disease despite at least 2 cycles of an intensive chemotherapy regimen designed to induce remission
- Refractory disease despite salvage therapy
- 2nd or greater relapse
- Any relapse after allogeneic hematopoietic cell transplantation
- 1st relapse if patient requires an allogeneic HCT as part of standard of care relapse therapy, but is found to be ineligible and/or unsuitable for HCT

*note: if patient met CD19+ ALL disease criteria, subsequent receipt of cancer directed therapy that eradicates disease does not preclude them from proceeding with this study

3.2.2 Age: ≤ 21 years of age

3.2.3 Karnofsky or Lansky (age-dependent) performance score ≥ 50 (Appendix A)

3.2.4 Estimated life expectancy of > 12 weeks

3.2.5 Meets eligibility criteria to undergo autologous apheresis, or have previously undergone autologous apheresis

3.3 Eligibility Criteria for Treatment with SJCAR19

3.3.1 Inclusion Criteria for Treatment with SJCAR19

3.3.1.1 $CD19^+$ ALL** with *any* of the following:

- Primary refractory disease despite at least 2 cycles of an intensive chemotherapy regimen designed to induce remission
- Refractory disease despite salvage therapy
- 2nd or greater relapse
- Any relapse after allogeneic hematopoietic cell transplantation
- 1st relapse if patient requires an allogeneic HCT as part of standard of care relapse therapy, but is found to be ineligible and/or unsuitable for HCT for any of the following reasons:

- Patients that do not have an available allogeneic donor (defined as at least a 7/8 HLA-matched related/unrelated donor, 5/6 HLA-matched umbilical cord donor, or 3/6 HLA-matched haploidentical donor)
- Patients with refractory leukemia, for which allogeneic transplant is known to be less effective in the B-ALL population, and
- Patients who are unable to receive myeloablative total body irradiation (TBI), which is included in standard transplant regimens for patients with B-ALL.

**ALL must be confirmed to be CD19+ within 3 months prior to enrollment for treatment

3.3.1.2 Detectable disease

3.3.1.3 Age: ≤ 21 years of age

3.3.1.4 Estimated life expectancy of > 8 weeks

3.3.1.5 Prior to planned SJCAR19 infusion, patients with a history of prior allogeneic HCT must be at least 3 months from HCT, have no evidence of active GVHD and have not received a donor lymphocyte infusion (DLI) within the 28 days prior to planned infusion

3.3.1.6 Adequate cardiac function defined as left ventricular ejection fraction > 40%, or shortening fraction ≥ 25%

3.3.1.7 EKG without evidence of clinically significant arrhythmia

3.3.1.8 Adequate renal function defined as creatinine clearance or radioisotope GFR ≥ 50 ml/min/1.73m^2 (GFR ≥ 40 ml/min/1.73m^2 if < 2 years of age)

3.3.1.9 Adequate pulmonary function defined as forced vital capacity (FVC) ≥ 50% of predicted value; or pulse oximetry ≥ 92% on room air if patient is unable to perform pulmonary function testing

3.3.1.10 Karnofsky or Lansky (age-dependent) performance score ≥ 50 (Appendix A)

3.3.1.11 Total Bilirubin ≤ 3 times the upper limit of normal for age, except in subjects with Gilbert's syndrome

3.3.1.12 Alanine aminotransferase (ALT) or aspartate aminotransferase (AST) ≤ 5 times the upper limit of normal for age

3.3.1.13 Has recovered from all NCI CTAE grade III-IV, non-hematologic acute toxicities from prior therapy

3.3.1.14 For females of child bearing age:

- Not lactating with intent to breastfeed
- Not pregnant with negative serum pregnancy test within 7 days prior to enrollment
- If sexually active, agreement to use birth control until 6 months after T-cell infusion. Male partners should use a condom

3.3.2 Exclusion Criteria for Treatment with SJCAR19

3.3.2.1 CNS-3 disease with or without neurologic changes

3.3.2.2 CNS-1/CNS-2 disease with neurologic changes

3.3.2.3 Known primary immunodeficiency

3.3.2.4 History of HIV infection

3.3.2.5 Evidence of active, uncontrolled neurologic disease

3.3.2.6 Severe, uncontrolled bacterial, viral or fungal infection

3.3.2.7 History of hypersensitivity reactions to murine protein-containing products

3.3.2.8 Receiving systemic steroids therapy exceeding the equivalent of 0.5 mg/kg/day of methylprednisolone, in the 7 days prior to CAR T-cell infusion (Appendix B)

3.3.2.9 Receiving systemic immunosuppressive therapy in the 14 days prior to CAR T-cell infusion, which will interfere with the activity of the SJCAR19 product in vivo (in the opinion of the study PI(s)).

3.3.2.10 Receiving intrathecal chemotherapy in the 7 days prior to CAR T-cell infusion

3.4 Research Participant Recruitment and Screening

This study will be posted on http://clinicaltrials.gov. Potential patients are typically referred from their primary clinical service at St. Jude, from the Physician Referral Office for outside St. Jude patients, or are currently being treated by the Department of BMTCT. Each patient considered for therapy is registered on the "Transplant List," an ongoing list used to provide the clinical service with information regarding new patients for consultation and under consideration for transplantation or cell therapy, and the status of those undergoing pre-evaluation. The patient remains on this list until a decision has been

confirmed for acceptance or rejection based on several factors, primarily completion of prior therapy and/or clinical status. The proposed patient schedules on this form are often subject to change due to various reasons such as family travel issues, donor availability for procurement of stem cells, recipient clinical status, etc. This list is a confidential document, updated and maintained by the Department of BMTCT Coordinators.

3.5 Enrollment on Study at St. Jude

A member of the study team will confirm potential participant eligibility as defined in Section 3.1-3.3, and register the participant in OnCore. Eligibility will be reviewed, and a research participant-specific consent form and assent document (where applicable) will be generated. The entire signed consent/assent form(s) must be either scanned and e-mailed to protocoleligibilityoffice@stjude.org or faxed to the Clinical Trials Operations (CTO) office at 901-595-6265 to complete the enrollment process. Refer to St. Jude SOP39 "Eligibility and Consent Verfication Processes."

To assist with enrollments and consent release, the CTO staff is available during office hours Monday – Friday. For help with patient enrollment after-hours, weekends, or holidays, please see the CTO intranet page for additional resources and instructions. Refer to St. Jude SOP CTO-TDOC-PE01 "Clinical Trials Management System Enrollment."

4.0 TREATMENT PLAN

4.1 Overview of treatment process

This protocol contains a 3-part consent process. Patients that meet eligibility for autologous apheresis will be consented to undergo collection of autologous peripheral blood mononuclear cells (PBMC) via apheresis. Alternatively, patients whom have previously undergone autologous apheresis may be considered for enrollment on the manufacturing portion of the study if it is determined that their previously collected product is appropriate for use in manufacturing; these patients would therefore not need to be consented using the SJCAR19 apheresis consent. When the patient meets eligibility criteria to begin manufacturing SJCAR19, they will be consented to proceed with manufacturing of the SJCAR19 product using their collected autologous cells. Lastly, when a SJCAR19 product has successfully been manufactured for an individual patient and they meet eligibility criteria to receive therapy with SJCAR19, they will be consented to proceed with the treatment portion of the study.

Collected apheresis cells may be cryopreserved, or used fresh to generate the SJCAR19 cell product. The SJCAR19 manufacturing process includes selection of T-cells from the apheresis product using a CliniMACS device, followed by activation and transduction of the selected T-cells. After transduction with the CD19-specific CAR lentivirus, T-cells will be expanded to the desired T-cell dose based on CAR^+ T-cells. The final SJCAR19 product will then be cryopreserved in treatment aliquots. After completion of quality assurance testing of SJCAR19, most patients will begin treatment with a lymphodepleting chemotherapy preparative regimen. This will be followed by a single infusion of SJCAR19, which will be administered thawed from frozen. Patients on the Phase II

study, who meet protocol defined criteria, may receive additional therapy with lymphodepleting chemotherapy and reinfusion of SJCAR19 (section 4.4.3).

See Appendix C for protocol flow chart. See Appendix D for a graphical summary of SJCAR19 manufacturing process.

4.2 Cell Procurement, Processing and Infusion

4.2.1 Autologous Apheresis and Storage

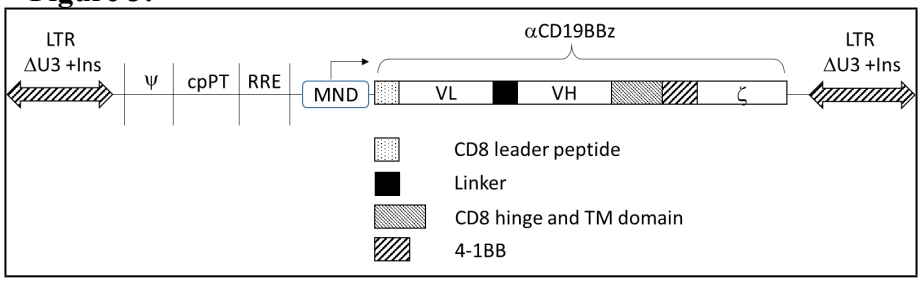
- Patients will undergo evaluation for suitability and eligibility for apheresis in accordance with departmental guidelines.
- The St. Jude Blood Donor Center of the Department of Pathology, accredited by both the American Association of Blood Bank (AABB) and Foundation for Accreditation of Cellular Therapy (FACT), and the Human Applications Laboratory of the Department of Therapeutic Production and Quality (TPQ), which is also FACT accredited, are responsible for the collection of the peripheral blood mononuclear cell (PBMC) product used in this protocol. Both groups are registered with the FDA and have established extensive quality assurance processes to ensure compliance with all Good Manufacturing Practice (GMP) and Good Tissue Practice (GTP) regulations. PBMC products will be collected as per current regulatory guidelines and established clinical practice procedures. Critical components include determination of donor eligibility, product tracking, manufacturing controls, documentation of lot production deviation investigations, product labeling, and release of products for use based on established lot criteria. Reporting of applicable deviations or events to the IRB and/or the FDA is coordinated with the PIs and St. Jude Office of Regulatory Affairs.
- The optimal timing of apheresis is not yet known. The timing of apheresis for each patient will be discussed with the collection team.
- It is recommended that patients undergo an unstimulated (no growth factors) collection of peripheral blood mononuclear cells (PBMC).
- It is suggested that prior to collection patients have:
  - An absolute lymphocyte count (ALC) $\geq 300/\mu L$
  - $CD3^+$ cell count $\geq 150/\mu L$
  - Not received systemic steroids therapy exceeding the equivalent of 0.5 mg/kg/day of methylprednisolone, in the 72 hours prior to apheresis
  - Not received systemic/intrathecal cytotoxic therapy in at least the 7 days prior to apheresis. When possible, the timing should be extended for those who have received longer acting agents such as peg-asparaginase
- The daily volume to be apheresed for PBMC collection is generally 3 − 4 total blood volumes.
- Collection goals:
  - The goal collection is 2×10^9 total nucleated cells (TNC) and/or 1×10^9 CD3+ cells

- Apheresis will generally be performed on a single day. Additional days of collection will be done at the discretion of a transplant physician based on goal collection criteria.
- Apheresis may be terminated early upon the request of a patient/guardian, or when deemed medically necessary per the judgment of study investigators.
- The collected PBMC will be processed and stored in single or multiple aliquots as per the standard operating procedures of the St. Jude Human Applications Laboratory.

4.2.2 CD19-specific CAR Lentiviral Production

A stable cell producer line for a lentivirus encoding CD19-CAR has been generated and vector lots are prepared by the St. Jude cGMP. The vector contains a chimeric antigen receptor consisting of a CD8 leader peptide, the CD19-specific single chain variable fragment (scFv) FMC63, the hinge and transmembrane domain of CD8α and the signaling domains of 4-1BB and CD3ζ under the control of a MND (myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted) promoter (Figure 3). Additionally, the 3' partially-deleted viral LTR includes a 400-bp fragment from the chicken hypersensitive site 4 chromatin insulator element. The vector is self-inactivating (SIN), derived from HIV.

Figure 3:



4.2.3 SJCAR19 Production

The starting material for the SJCAR19 manufacturing process is an autologous apheresis product containing peripheral blood mononuclear cells (PBMC). The PBMC are used fresh, or after being thawed and washed. T-cells are then selected using Militenyi's CD8 and CD4 reagents using the CliniMACS™ system. The positively selected T-cells are then activated with GMP grade CD3 and CD28 monoclonal antibodies or agonists, transduced with CD19-specific CAR lentiviral vector and expanded using cytokines. After the expansion period, the transduced T-cells are harvested, washed and pooled. After sampling for QC testing, the transduced T-cells are formulated for the cell dose in cryoprotectant and the cells are cryopreserved using a controlled rate freezer. The cryopreserved cells are stored in the vapor phase of liquid nitrogen. If more than the required number of i) cells are collected for SJCAR19 production, or ii) SJCAR19 cells are produced, detailed phenotypic and functional analyses as outlined in section 6.1.3 will be performed. In addition, samples might be used for studies to improve current cell processing procedures.

4.2.4 Quality Assurance of the Cellular Product

Products that meet study specific release criteria, as detailed on the Certificate of Analysis (CoA), will be infused as per Section 4.4.

4.3 Bridging Chemotherapy

- While the SJCAR19 cell product is being manufactured and released for clinical use, subjects may receive best clinical management bridging chemotherapy at the discretion of the primary oncologist in close consultation with a PI of this study. Bridging chemotherapy will be defined in a separate document requiring additional consent.
- When choosing a regimen for an individual patient, the goal should be that the patient be expected to recover from any toxicities within 3 weeks of receipt of the bridging chemotherapy regimen.
- Patients cannot receive intrathecal (IT) chemotherapy within 7 days of receipt of SJCAR19 product.
- Patients should not receive blinatumomab as part of their bridging chemotherapy regimen.
- Patients will be required to undergo repeat disease evaluation after completion of bridging chemotherapy, prior to beginning treatment with SJCAR19

4.4 SJCAR19 Therapy

4.4.1 SJCAR19 Treatment Schedule (initial infusion)

On both the Phase I and Phase II portions of the clinical study, patients will receive lymphodepleting chemotherapy* followed by infusion of SJCAR19 (dosing in section 4.4.2). See Table 1 for example treatment schedule.

Table 1: SJCAR19 Treatment Schedule (initial infusion)

DAY	MEDICATION*	DOSE	DOSE #
-4	Fludarabine	25 mg/m^2 **	1 of 3
-3	Fludarabine	25 mg/m^2 **	2 of 3
-2	Fludarabine	25 mg/m^2 **	3 of 3
	Cyclophosphamide	900 mg/m^2 **	1 of 1
-1	REST DAY		
0 or +1	SJCAR19 Infusion	Assigned dose	1 of 1

*For patients that have contraindications to receiving fludarabine and/ or cyclophosphamide, in discussion with the PI of the study they may receive no lymphodepleting chemotherapy or an alternative regimen

**dose adjustment for patients ≤ 10kg: Fludarabine 0.83 mg/kg/dose and Cyclophosphamide 30 mg/kg

General treatment related comments:

- The term "day" does not refer to an absolute calendar day. It refers to a general 24-hour period.
- Dosing for the medications cyclophosphamide and fludarabine may be modified for research recipients based upon actual body weight or adjusted ideal body weight when clinically indicated. Mesna will be administered for prevention of hemorrhagic cystitis.

- Criteria for medication calculations based on body weight/body surface area and other medication related information can be found in the St. Jude Formulary http://www.crlonline.com/crlsql/servlet/crlonline or the St. Jude Department of Pharmaceutical Sciences intranet website http://home.web.stjude.org/pharmaceutical_ser/drugInfo.shtml. Medication doses may be rounded to the nearest integer or to the nearest appropriate quantity when clinically or pharmaceutically indicated as per the MD and PharmD.

4.4.2 SJCAR19 Dosing and Initial Infusion

General treatment related comments:

- Dosing of SJCAR19 is determined based on the number of CAR^+ T cells in the SJCAR19 product and the patient weight in kilograms, with a maximum dose of 2.5×10^8 CAR^+ T cells.
- SJCAR19 infusion will occur at least 36 hours after the completion of lymphodepleting chemotherapy.
- At the time of SJCAR19 infusion, the subject should be afebrile and without active, uncontrolled infection. If fever is not responsive to anti-pyretics, and not due to an identifiable infectious etiology, the patient may receive the SJCAR19 infusion in consultation with the study PI(s).
- At the time of SJCAR19 infusion, the subject should be without evidence of rapidly progressive leukemia
- Patients should be pre-medicated with Benadryl and/or Tylenol (dosing per institutional formulary) prior to infusion of SJCAR19, unless there is a medical contraindication against using either of these medications.
- SJCAR19 will be given thawed from frozen, as a single intravenous (IV) infusion.
- For the proper infusion procedures and monitoring of the SJCAR19 product please refer to BMTCT SOP 40.04. Please note that all relevant SOPs can be found on the BMTCT Clinical Transplant Program intranet page: https://home.stjude.org/bmt/Pages/policies-transplant-program.aspx
- All patients shall receive the SJCAR19 infusion while inpatient. In the Phase I portion of the study, patients will remain inpatient for at least 7 days post-infusion; in the Phase II portion of the study, patients will remain inpatient for at least 3 days post-infusion. At that time, discharge will be determined based on current clinical status. All patients shall remain locally until completion of week 4 study evaluations.

Phase I treatment related comments:

- On the Phase I portion of the study, up to 3 different dose levels will be evaluated (see Table 2), with a maximum dose of 2.5×10^8 CAR^+ T cells.

- Escalation and de-escalation between dose levels will be determined by protocol defined dose-limiting toxicities (DLTs, see section 11.1).

Table 2: Phase I Dose Level Escalation of SJCAR19*

Dose Level	SJCAR19 Dose** (Cells/kg)
-1	0.3×10^6
1	1×10^6
2	3×10^6

* +/- 20%
** the maximum dose of SJCAR19 is 2.5×10^8 CAR^+ T cells

Phase II treatment related comments:

- On the Phase II portion of the study, all patients will receive the Phase I determined maximum tolerated dose (MTD) of SJCAR19, with a maximum dose of 2.5×10^8 CAR^+ T cells.

4.4.3 Optional Reinfusion of SJCAR19 for Phase II Study Participants

Phase II study participants that remain on-therapy and meet specified criteria will have the option to receive additional treatment courses. Treatment will consist of lymphodepleting chemotherapy and infusion of SJCAR19 cells, using cryopreserved manufactured product that was generated in excess of what was needed for the initial infusion. Patient may receive multiple treatment courses.

4.4.3.1 Criteria for subsequent SJCAR19 infusions

- Autologous B-cell recovery within 6 months of prior infusion, defined as:
  - $\geq$ 1% CD19+ cells in peripheral blood at 2 consecutive timepoints, at least 1 week apart

  AND/OR
  - An absolute CD19+ count of $\geq$ 50/uL at a single timepoint

AND/OR

- Recurrence of detectable leukemia within 12 months of prior infusion, defined as:
  - < 5% morphologic blasts AND at least $\geq$ 0.01% blasts by flow cytometry, $\geq 10^{-4}$ by PCR, and/or $\geq 10^{-5}$ by NGS

  OR
  - $\geq$ 5% morphologic blasts with confirmation of disease by flow, RT-PCR and/or NGS

- If a participant is eligible for reinfusion based on one of the above two criteria, they must also meet the following conditions: For those with detectable leukemia: confirmation of CD19+ expression on leukemia blasts (exception: not feasible for those with disease detectable by only RT-PCR or NGS)
- Prior complete response (MRD-positive or MRD-negative) to SJCAR19 infusion as defined in Table 4
- At least 4-weeks since previous cell infusion
- Meet all initial organ-function related eligibility criteria as outlined in section 3.3
- Available cryopreserved SJCAR19 product at appropriate dosing

4.4.3.2 Treatment (Reinfusion)

- Participants may receive additional bridging therapy prior to reinfusion infusion of SJCAR19; regimen will be per treating physician discretion (section 4.3). While bridging therapy will not be given as part of the SJCAR19 study, patients will remain on-therapy/on-study.

- Treatment will follow section 4.4 with the following exceptions:
  - Dosing of SJCAR19 reinfusion shall not be greater than the protocol defined MTD
  - Participants will receive intensified lymphodepletion using the regimen below (Table 3); alternatively, in discussion with the PI the regimen may instead follow that as outlined in Table 1.
  -

Table 3: Reinfusion: Intensified Lymphodepletion Regimen

DAY	MEDICATION*	DOSE**	DOSE #
-4	Fludarabine	40 mg/m^2	1 of 3
-3	Fludarabine	40 mg/m^2	2 of 3
	Cyclophosphamide	600 mg/m^2	1 of 2
-2	Fludarabine	40 mg/m^2	3 of 3
	Cyclophosphamide	600 mg/m^2	2 of 2
-1	REST DAY		
0	SJCAR19 Reinfusion***	MTD	1 of 1

*For patients that have contraindications to receiving fludarabine and/or cyclophosphamide, in discussion with the PI of the study they may receive no lymphodepleting chemotherapy or an alternative regimen

**dose adjustment for patients ≤ 10kg: Fludarabine 1.3 mg/kg/dose and Cyclophosphamide 20 mg/kg

***Phase II cell dose: 3 x 10^6 CAR+ T cells/kg (+/- 20%); the maximum dose of SJCAR19 is 2.5 x 10^8 CAR+ T cells

4.4.3.3 Post-infusion monitoring

- For each reinfusion treatment course, post-infusion monitoring will 'start over', following that as outlined for 1st infusions on the Phase II study. This includes but is not limited to:
  - Treatment evaluations (section 6.0; see also Appendices E and I; starting at week -1 time point)
  - Response evaluations (section 7.0)
  - Adverse event recording and reporting (section 9.0)

- Additionally, if two or more participants develop Grade 4 toxicity at any time following reinfusion, that is felt to be possibly, probably or likely related to the SJCAR19 product, then reinfusions will be paused pending discussion with the FDA and IRB regarding continuing to allow reinfusions.

4.4.3.4 Statistical considerations (Reinfusion):

Data from reinfusion may be included in safety, efficacy and exploratory analysis. This data will be collected as a unique infusion, adding additional data points, but not as a unique patient (i.e. reinfusion does not count towards enrollment goals).

4.5 Supportive Care

4.5.1 Venous Access

Chemotherapy and SJCAR19 will be given intravenously, either centrally or peripherally.

4.5.2 Allergic Reactions

Standard medications to treat allergic reactions should be available at the bedside prior to infusion of SJCAR19 (see BMTCT SOP 40.04).

4.5.3 Blood Product Support

Blood products are to be administered based on clinical status and the BMTCT SOP 50.06.

4.5.4 Fever and Neutropenia

Standard of care should be administered for complications resulting from neutropenia. Please refer to the St. Jude Institutional "Febrile Neutropenia in Patients with Cancer" Guidelines, which can be located on the St. Jude Intranet.

4.5.5 Cytokine Release Syndrome

Cytokine release syndrome (CRS) is a commonly reported toxicity with treatment with CD19-specific CAR-T cells. Patients will be closely monitored for signs and symptoms of CRS, and treatment guided by BMTCT SOP 20.15. The goal of interventions should be to prevent life-threating consequences of CRS, while attempting to preserve the anti-tumor benefits of SJCAR19.

4.5.6 Neurotoxicity
Neurotoxicity is a commonly reported side effect of treatment with CD19-specific CAR-T cells. Patients will be closely monitored for signs and symptoms of neurotoxicity, and treatment guided by BMTCT SOP 20.15. The goal of interventions should be to prevent life-threating consequences of neurotoxicity, while attempting to preserve the anti-tumor benefits of SJCAR19.

4.5.7 Hypogammaglobinemia
During the period of B-cell aplasia, supportive care will include giving participants intravenous immunoglobulins (IVIG) if their IgG level is lower than 400 mg/dl.

4.6 Concomitant therapy and/or enrollment onto therapeutic protocols post-infusion of SJCAR19
Participants will not be allowed to receive concomitant therapy for primary disease or high dose steroids (> 0.5 mg/kg/day methylprednisolone equivalent; see Appendix B) during the first 4 weeks after SJCAR19 infusion. A disease status evaluation will be conducted approximately 4 weeks post-SJCAR19 infusion. If at this time complete response is not achieved, the participant will be allowed to receive chemotherapy or enroll onto a therapeutic study for their disease if available.

5.0 DRUG/DEVICE/BIOLOGIC AGENT INFORMATION

5.1 Medications

Cyclophosphamide (Cytoxan)	
Source & Pharmacology	Cyclophosphamide is a nitrogen mustard derivative. It acts as an alkylating agent that causes cross-linking of DNA strands by binding with nucleic acids and other intracellular structures, thus interfering with the normal function of DNA. It is cell cycle, phase non-specific. Cyclophosphamide is well absorbed from the GI tract with a bioavailability of >75%. It is a prodrug that requires activation. It is metabolized by mixed function oxidases in the liver to 4-hydroxycyclo-phosphamide, which is in equilibrium with aldophosfamide. Aldofosfamide spontaneously splits into nitrogen mustard, which is considered to be the major active metabolite, and acrolein. In addition, 4-hydroxycy-clophosphamide may be enzymatically metabolized to 4-ketocyclophosphamide and aldophosfamide may be enzymatically metabolized to carboxyphosphamide that is generally considered inactive. Cyclophosphamide and its metabolites are excreted mainly in the urine. Dose adjustments should be made in patients with a creatinine clearance of <50 ml/min.
Formulation and Stability	Cyclophosphamide is available in vials containing 100, 200, 500, 1000 and 2000mg of lyophilized drug and 75 mg mannitol per 100 mg of cyclophosphamide. Both forms of the drug can be stored at room temperature. The vials are reconstituted with 5, 10, 25, 50 or 100 ml of sterile water for injection, respectively, to yield a final concentration of 20 mg/ml. Reconstituted solutions may be further diluted in either 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically stable for 24 hours at room

	temperature and 6 days if refrigerated, but contain no preservative, so it is recommended that they be used within 24 hours of preparation.
Supplier	Commercially available
Toxicities	Dose limiting toxicities of cyclophosphamide includes BM suppression and cardiac toxicity. Cardiac toxicity is typically manifested as congestive heart failure, cardiac necrosis or hemorrhagic myocarditis and can be fatal. Hemorrhagic cystitis may occur and necessitates withholding therapy. The incidence of hemorrhagic cystitis is related to cyclophosphamide dose and duration of therapy. Forced fluid intake and/or the administration of Mesna decreases the incidence and severity of hemorrhagic cystitis. Other toxicities reported commonly include nausea and vomiting (may be mild to severe depending on dosage), diarrhea, anorexia, alopecia, immunosuppression and sterility. Pulmonary fibrosis, SIADH, anaphylaxis and secondary neoplasms have been reported rarely.
Route	Intravenous infusion

Fludarabine (Fludara)	
Source & Pharmacology	Fludarabine phosphate is a synthetic purine nucleoside analog. It acts by inhibiting DNA polymerase, ribonucleotide reductase and DNA primase by competing with the physiologic substrate, deoxyadenosine triphosphate, resulting in inhibition of DNA synthesis. In addition, fludarabine can be incorporated into growing DNA chains as a false base, thus interfering with chain elongation and halting DNA synthesis. Fludarabine is rapidly dephosphorylated in the blood and transported intracellularly via a carrier-mediated process. It is then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate form. Approximately 23% of the dose is excreted as the active metabolite in the urine (with dosages of 18-25 mg/m^2/day for 5 days). Renal clearance appears to become more important at higher doses, with approximately 41-60% of the dose being excreted as the active metabolite in the urine with dosages of 80-260 mg/m^2.
Formulation and Stability	Fludarabine is supplied in single-dose vials containing 50 mg fludarabine as a white lyophilized powder and 50 mg of mannitol. The intact vials should be stored under refrigeration. Each vial can be reconstituted by adding 2 ml of sterile water for injection resulting in a final concentration of 25 mg/ml. Because the reconstituted solution contains no antimicrobial preservative, the manufacturer recommends that it should be used within 8 hours of preparation. The solution should be further diluted in 5% dextrose or 0.9% NaCl prior to administration.
Supplier	Commercially available.
Toxicities	The major dose-limiting toxicity of fludarabine is myelosuppression. Nausea and vomiting are usually mild. Side effects reported commonly include anorexia, fever and chills, alopecia and rash. Neurotoxicity can be manifested by somnolence, fatigue, peripheral neuropathy, mental status changes, cortical blindness and coma and is more common at high doses. Neurotoxicity is usually delayed, occurring 21-60 days after the completion of a course of therapy and may be irreversible. Side effects reported less commonly include diarrhea, stomatitis, increased liver function tests, liver failure, chest pain, arrhythmias and seizures. Pulmonary toxicity includes allergic pneumonitis characterized by cough, dyspnea, hypoxia and pulmonary infiltrates. Drug induced pneumonitis is a delayed effect, occurring 3-28 days after the administration of the third or later

	course of therapy. Administration of corticosteroids usually results in resolution of these symptoms.
Route	Intravenous

Mesna (Mesnex)	
Source & Pharmacology	Mesna is a synthetic sulfhydryl (thiol) compound. Mesna contains free sulfhydryl groups that interact chemically with urotoxic metabolites of oxaza-phosphorine derivatives such as cyclophosphamide and ifosfamide. Oral bioavailability is 50%. Upon injection into the blood, Mesna is oxidized to Mesna disulfide, a totally inert compound. Following glomerular filtration, Mesna disulfide is rapidly reduced in the renal tubules back to Mesna, the active form of the drug. Mesna and Mesna disulfide are excreted primarily via the urine.
Formulation and Stability	Mesna is available in 2 ml, 4 ml and 100 ml amps containing 100 mg/ml of Mesna solution. The intact vials can be stored at room temperature. Mesna may be further diluted in 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically and chemically stable for at least 24 hours under refrigeration.
Supplier	Commercially available
Toxicities	Mesna is generally well tolerated. Nausea and vomiting, headache, diarrhea, rash, transient hypotension and allergic reactions have been reported. Patients may complain of a bitter taste in their mouth during administration. Mesna may cause false positive urine dipstick readings for ketones.
Dosage and Administration	Mesna is generally dosed at approximately 25% of the cyclophosphamide dose. It is generally given intravenously prior to and again at 3, 6 and 9 hours following each dose of cyclophosphamide.
Route	Intravenous

5.2 CliniMACS™ System

The mechanism of action of the CliniMACS Cell Selection System is based on magnetic-activated cell sorting (MACS). The CliniMACS device is a powerful tool for the isolation of many cell types from heterogeneous cell mixtures, (e.g. apheresis products). These can then be separated in a magnetic field using an immunomagnetic label specific for the cell type of interest, such as $CD4^+$ or $CD8^+$ human T cells. The basic mechanism is the same for either application; target cells are "tagged" with super-paramagnetic particles and eventually separated from the unlabeled cells using the CliniMACS device as described above. The desired target cells can either be infused or discarded appropriately. The CliniMACS device is not licensed by the FDA for CD4 or CD8 selection, and therefore is investigational.

The CliniMACS device is comprised of a computer controlled instrument incorporating a strong permanent magnet, a closed-system sterile tubing set containing columns with a coated ferromagnetic matrix and a paramagnetic, cell specific, labeling reagent. Cells are labeled with antibody-conjugated beads that target a surface maker of interest. The monoclonal antibody is conjugated to a super-paramagnetic particle that is small in size (about 50 nm in diameter) and composed of iron oxide/hydroxide and dextran. These magnetic particles form a stable colloidal solution and do not precipitate or aggregate in magnetic fields. The antibody conjugated beads used in this system are highly specific

(e.g. $CD4^+$ and $CD8^+$ conjugated beads). High-gradient MACS technology has been shown to achieve rapid and highly specific depletion or enrichments of large numbers of target cells from bone marrow, cord blood, and normal peripheral blood mononuclear cells. The CliniMACS device incorporates a strong permanent magnet and a separation column with a ferromagnetic matrix to separate the cells labeled with the magnetic particles. The high-gradient system allows the application of strong magnetic forces and a rapid de-magnetization. Small ferromagnetic structures, such as the column matrix, placed in a magnetic field concentrate this homogenous field and thereby produce high magnetic gradients. In their immediate proximity, the ferromagnetic structures generate magnetic forces 10,000-fold greater than in the absence of those structures enabling the retention of magnetically labeled cells. After removing the column from the magnet, the rapid de-magnetization of the column matrix allows the release of retained cells.

6.0 REQUIRED EVALUATIONS, TESTS, AND OBSERVATIONS

At the time of autologous apheresis, information regarding the patient's current clinical status will be recorded. Studies looking at the immunologic content of the apheresis and SJCAR19 products will be performed.

Once the patient proceeds with the treatment portion of the protocol, and for all reinfusions, a complete history, physical examination, and performance status is necessary prior to lymphodepletion (week -1) and SJCAR19 administration (week 0). A complete history, physical exam and performance status will be collected at week 1, 2, 3, 4, and 8. A complete history will continue to be collected every three months for a year. Starting week 8, contact by the investigator or research nurse/research coordinator by phone or email may substitute for history if the patient is unable to return to clinic. The week 8 physical examination and performance score can also be completed by the patient's local treatment team if the patient is unable to return to St. Jude for evaluation.

All patients shall receive the SJCAR19 infusion while inpatient. In the Phase I portion of the study, patients will remain inpatient for at least 7 days post-infusion; in the Phase II portion of the study (for initial and reinfusions), patients will remain inpatient for at least 3 days post-infusion. At that time, discharge will be determined based on current clinical status. <u>All patients shall remain locally until completion of week 4 study evaluations</u>.

6.1 <u>Treatment Evaluations</u>

All evaluations are summarized in the study calendar provided in Appendix E. Daily maximum blood draw will follow institutional standards.

6.1.1 <u>Standard Laboratory Investigations</u>

- CBC and differential: pre-lymphodepleting chemotherapy (wk -1), post-lymphodepleting chemotherapy but pre-SJCAR19 infusion (wk 0), at 1, 2, 3, 4 and 8 weeks and then at 3, 6, 9, and 12 months post-SJCAR19 infusion.

- BUN, Creatinine, Na^+, K^+, Cl^-, CO_2, Mg, P: pre-lymphodepleting chemotherapy (wk -1), post-lymphodepleting chemotherapy but pre-SJCAR19 infusion (wk 0), at 1, 2, 3, 4 and 8 weeks post-SJCAR19 infusion.

- AST/ALT, Bilirubin, Albumin: pre-lymphodepleting chemotherapy (wk -1), post-lymphodepleting chemotherapy but pre-SJCAR19 infusion (wk 0), at 1, 2, 3, 4 and 8 weeks post-SJCAR19 infusion.

- CRP, Ferritin: pre-lymphodepleting chemotherapy (wk -1), post-lymphodepleting chemotherapy but pre-SJCAR19 infusion (wk 0), at 1, 2, 3, 4 and 8 weeks post-SJCAR19 infusion.

- Lymphocyte Subset Panel: pre-lymphodepleting chemotherapy (wk -1), at 4 and 8 weeks and then at 3, 6, 9, and 12 months post-SJCAR19 infusion.

- Quantitative Immunoglobulins: pre-lymphodepleting chemotherapy (wk -1), at 4 and 8 weeks and then at 3, 6, 9, and 12 months post-SJCAR19 infusion.

- Serum pregnancy test will be performed within the week preceding starting pre-lymphodepleting chemotherapy (wk -1) on female patients of child bearing potential unless they have no possibility of pregnancy (for example post hysterectomy).

- Other tests: Infectious disease testing will be done at the time of blood procurement for SJCAR19 production.

- Additional routine laboratory investigations may be performed as per institutional guidelines during the administration of lymphodepleting chemotherapy.

6.1.2 Other Laboratory Testing

- Serum from blood drawn at week 0 and week 4 will be stored for measurement of Human anti-mouse antibodies (HAMA) in the event of a suspected immunologic reaction.

6.1.3 SJCAR19 Research Laboratory Testing

All of these tests may not be performed on every specimen as a limited number of cells will be available. In general, priority will be given to PCR assays to detect for the presence of SJCAR19. Additional samples may be sent if the patient has concern for disease relapse/progression, or an acute clinical change that is felt to possibly be related to the SJCAR19 product (see section 6.1.6). Furthermore, research laboratory testing may also be performed using a leftover clinically indicated sample.

SJCAR19 Research Labs:
Persistence/Functional studies:

- Quantitative real-time PCR
- FACS analysis
- If detection of SJCAR19 is greater than 0.5% via FACS and there is persistent SJCAR19 expansion 3 months after T-cell infusion, we will perform clonality studies (an extra 5 ml of blood). Further evaluations may be done based on clinical decisions.
- Persistence and/or Functional studies may include studies such as: phenotypic subset characterization, functional correlates (e.g. Elispot assay, PCR to determine viral load), methylation analysis, multipotency indices, analysis of the epigenetic signatures, TCR repetiore analysis, VIS analysis and single cell analysis such as RNA seq.

Persistence and/or Functional Studies may be performed on peripheral blood, bone marrow aspirate, or CSF to monitor function, persistence and safety of SJCAR19 and unmodified immune cells at time-points indicated in the study calendar.

Samples to be obtained on peripheral blood pre-lymphodepleting chemotherapy (wk -1), post-lymphodepleting chemotherapy but pre-SJCAR19 infusion (wk 0), 1 to 4 hours post infusion, 3 - 4 days post infusion (optional per discretion of PI/Transplant Attending), at 1, 2, 3, 4 and 8 weeks, and then at 3, 6, 9, and 12 months.

Cytokine studies:

- Samples for serum or plasma cytokines/chemokines levels will be obtained, and stored, at the following time points: pre-lymphodepleting chemotherapy (wk -1), post-lymphodepleting chemotherapy but pre-SJCAR19 infusion (wk 0), 1 to 4 hours post infusion, 3 - 4 days post infusion (optional per discretion of PI/Transplant Attending), and at 1, 2, 3, 4 and 8 weeks. Beginning at wk 2, analysis will be optional based on the clinical course of each patient (per discretion of the study PIs).

Microenvironment and leukemia blast studies:
For evaluation of tumor microenvironment and leukemia blasts, we will collect a sample at the 4-week post-CAR T-cell infusion disease evaluation. If a patient has circulating peripheral blood blasts at any time while on study, this analysis may also be performed on that study sample. Analysis may also be performed on prior banked tumor samples/diagnostic samples, including the most recent marrow sample pre-CAR therapy, or through comparison to existing genomic data.

- Analysis for microenvironment and leukemia blast studies may include: flow cytometry, single cell RNAseq, and genomic, transcriptomic and proteomic analysis.

6.1.4 Other Testing

- To assess cardiac function, an echocardiogram (ECHO) and EKG will be performed within 2 weeks of starting lymphodepleting chemotherapy regimen.
- An additional EKG will be performed post-lymphodepleting chemotherapy but pre-SJCAR19 infusion (wk 0).

6.1.5 Evaluation for Leukemic Disease

Minimal residual disease (MRD) assays will be performed in the bone marrow, cerebrospinal fluid (CSF) and/or peripheral blood by immunologic and molecular methods. For all patients, MRD will be assessed for by flow cytometry if they have MRD that is able to be followed by flow. For those patients that have disease that is able to be followed by PCR testing, this testing will also be performed, either in lieu of flow if flow is not available, or in addition to flow cytometry methods. Tests will be performed using flow, PCR and/or next generation sequencing (NGS).

- Peripheral blood MRD: within 2 weeks prior to start of lymphodepleting chemotherapy (after receipt of any bridging chemotherapy and before protocol pre-lymphodepleting chemotherapy), post-lymphodepleting chemotherapy but pre-SJCAR19 infusion (wk 0), and at 4 weeks and 3 months post SJCAR19 infusion.

- Bone marrow aspirates: within 2 weeks prior to start of lymphodepleting chemotherapy (after receipt of any bridging chemotherapy and before protocol pre-lymphodepleting chemotherapy), and at 4 weeks and 3 months post-SJCAR19 infusion.

- CSF: within 2 weeks prior to start of lymphodepleting chemotherapy (after receipt of any bridging chemotherapy and before protocol pre-lymphodepleting chemotherapy) and at 4 weeks post-SJCAR19 infusion. For patients with recent history of CNS disease, sample also to be obtained at 3 months post-SJCAR19 infusion.

- For patients who get additional clinical evaluations related to monitoring for leukemic disease (such as PET-CT or biopsy), results will be recorded.

6.1.6 Other tissue samples

For any additional tissue sample that is obtained while the patient is on this study to determine their diseases status, we will obtain an extra sample for testing as described in section 6.1.3.

- For any patients who die or develop a second neoplasm, significant hematologic or neurologic disorder during the trial, a biopsy sample of the neoplastic tissue or pertinent autopsy tissue will be assayed by PCR for presence of the SJCAR19 transgene (if a sample can be obtained).

6.1.7 Evaluation for RCL

RCL testing will be performed pre-lymphodepleting chemotherapy (wk -1) and 3, 6, and 12 months post-SJCAR19 infusion.

6.2 Patient Reported Outcomes and Quality of Life Evaluations

All questionnaires aimed at assessing patient QoL can be administered directly to the patient or to a caregiver proxy. We will attempt to obtain patient data at every opportunity, i.e. when the patient is of an appropriate age to complete the measure based on prior validation studies and when the patient is medically able to complete the measure. We will also attempt to obtain proxy data for each questionnaire at each time point in anticipation of the likelihood that a significant proportion of patients may be medically unable to participate at one or more time points. Having consistent proxy measures will allow for ease of longitudinal comparison over time. Data will be obtained from patients and caregivers independently such that if one or the other is unable to participate, data can still be collected from the available participant. For Spanish-speaking participants, validated Spanish language questionnaires will be administered when available. If a validated Spanish questionnaire is not available, the questionnaire will not be administered.

The semi-structured interviews will be conducted with English-speaking patients and caretakers. Both parties will be engaged in the questions and answers from all participants will be recorded and transcribed for analysis. Semi-structured interviews will not be conducted with non-English-speaking particpants.

All components of the quality of life evaluation can be administered over the phone. Every effort will be made to obtain questionnaire data and perform semi-structured interviews in person, but if this is not feasible all data can be collected by phone.

All evaluations are summarized in the study calendar provided in Appendix I.

6.2.1 Symptom Assessment

PRO-CTCAE, Core Terms: This is a 15-item Likert-type scale developed to assess adverse events, their severity/frequency, and the degree of consequent functional impairment. For participants < 7 years of age, we will ask that the primary caretaker complete the Proxy PRO-CTCAE. Participants 7 to 15 years of age will complete the Pediatric PRO-CTCAE, with help of research staff as needed. Participants aged 16 years or older will complete the Adult PRO-CTCAE. This measure is available in both English and Spanish.

6.2.2 Evaluation of Patient Quality of Life

The Pediatric Quality of Life Inventory (PedsQL V.4): This is a 23-item Likert-

type scale developed to measure health-related QOL in children and adolescents. It takes about 4 to 6 minutes to complete. This instrument has a form for caregivers, including both the acute (QoL within the past 7 days) and chronic (QoL within the past 30 days) forms. Subscales on the PedsQL V.4 measure the research participant's physical, emotional, social, and school functioning. The forms have been found to be internally consistent (Cronbach alpha coefficients of 0.92). They were also shown to have clinical validity (i.e., able to distinguish between known groups of research participants on and off therapy), and to allow construct validity (i.e., hypotheses predicting relationships between certain subscales and other indicators of emotional distress, perceived competency, social support/functioning, and academic competence were supported). This measure is available in both English and Spanish.

The PedsQL Cancer Module V.3: This is a 27-item Likert-type questionnaire that involves 8 subscales relevant to the pediatric cancer experience: 1) pain and hurt, 2) nausea, 3) procedural anxiety, 4) treatment anxiety, 5) worry, 6) cognitive problems, 7) perceived physical appearance, and 8) communication. This measure is available in both English and Spanish.

Good Day Bad Day Scale: This is a qualitative instrument that will ask two questions to the research participants: "What makes a good day for you?" and "How has being sick been for you?" This semi-structured interview will only be conducted with English-speaking participants.

Qualitative Symptoms Assessment: Through semi-structured interviews, patients will also be asked to identify their most bothersome or worrying symptoms. Trained interviewers will then probe with follow-up questions to understand exactly what symptoms patients are experiencing, the severity of these symptoms, and to what degree they interfere with daily function. Interview will also probe what patient and caregivers recall from earlier consent conversations re: study participation and signs and symptoms. We will explore whether anticipatory guidance about side effects and risks was helpful for patients that went on to experience those side effects. This semi-structured interview will only be conducted with English-speaking participants.

6.2.3 Evaluation of Caretaker Well-being and Quality of Life

The Caregiver Quality of Life Index – Cancer (CQOLC)[82]*:* This is a 35-item questionnaire to assess QoL of the family caregiver of each research participant. It can be completed in 10 minutes. The items assess the caregiver's overall mental health, emotional distress, physical health, social support, social desirability, as well as the research participant's performance status and related caregiver QoL. The instrument has been found to have adequate validity, test-retest reliability (0.95) and internal consistency (Cronbach's alpha coefficient of 0.91). This measure is available in both English and Spanish.

The Functional Assessment of Chronic Illness Therapy – Spiritual Well-Being (FACIT-Sp-12)[83]*:* This is a 12-item Likert-type scale to measure one's sense of meaning, harmony, peacefulness, and a sense of comfort and strength. It requires

5 to 6 minutes to be completed. It has been found to have good psychometric properties in assessing the spiritual well-being of people with cancer and their caregivers and is the most commonly used clinical research tool to assess spiritual well-being, an important factor in overall and health-related quality of life. This measure is available in English and Spanish.

The Hospital Anxiety and Depression Scale[84]: This is a 14-item Likert scale developed to assess the cognitive symptoms of anxiety and depression. Psychometric properties of this scale have supported the use of this tool among research participants and family caregivers. The internal consistency of the anxiety and depression scale was good (Cronbach's alpha coefficients of 0.83 and 0.82). This scale was also sensitive and specific. It can be completed in 6-7 minutes. This measure is only available in English.

The Herth Hope Index (HHI)[84, 85]*:* This is a 12-item Likert scale developed to assess hope in adults in clinical settings. The scale is adapted from the Herth Hope Scale (HHS) and has a Cronbach's alpha coefficient of 0.97 with a two – week test – retest reliability of 0.91. Criterion-related validity ranges from r = 0.92 to r = 0.73. This scale has been used extensively in the clinical setting. It can be administered in 5 to 6 minutes and can be scored easily and reliably. This measure is available in English and Spanish.

The Modified Parent-Patient Activation Measure (mP-PAM)[86]*:* This is a 13-item Likert-like scale developed to assess degree of caregiver knowledge, skill, and confidence for self-management. The internal consistency of this scale is good (Cronbach's alpha coefficient of 0.90 in English-speaking populations). It has been modified from its original version to address issues facing the pediatric cancer population. This measure is only available in English.

6.3 Long-term Follow-up Evaluations

Long-term follow-up will occur for 15 years as per FDA guidance. Follow-up will occur on protocol for the first year after the last SJCAR19 infusion. After one year, patients will be approached and consented to the current institutional long-term follow-up protocol for patients treated with genetically modified cell therapy products.). If a patient does not enroll on this long-term follow-up protocol, we will continue to monitor patients on-study, for up to 15 years post-infusion, per current FDA guidance ('*Long Term Follow-Up after Administration of Human Gene Therapy Products*' and '*Testing of Retroviral Vector-Based Human Gene Therapy Products for Replication Competent Retrovirus During Product Manufacture and Patient Follow-up*').

7.0 EVALUATION CRITERIA

7.1 Toxicity Evaluation Criteria

Adverse event (AE) monitoring for on-study research participants will be assessed using the NCI Common Terminology Criteria for Adverse Events Version 5.0. The standard procedures for adverse event collection and monitoring are noted in Appendix F. An

exception will be made with the staging and grading of CRS and neurotoxicity, which will be assessed and graded using the ASTCT consensus grading system (Appendix G and H)..

7.2 Disease Response Criteria

Disease response criteria will based on the level of disease a patient has prior to treatment with SJCAR19. Response will be determined at 4 weeks post-SJCAR19 infusion based on disease status in a bone marrow sample. For all patients, MRD will be assessed for by flow cytometry if they have MRD that is able to be followed by flow. For those patients that have disease that is able to be followed by PCR testing or NGS testing, this testing will also be performed, either in lieu of flow if flow is not available, or in addition to flow cytometry methods. MRD is defined as leukemia detection via flow of $\geq$ 0.01%, PCR of $\geq 10^{-4}$, and/or NGS of $\geq 10^{-5}$. See Table 4 for disease response criteria.

Table 4: Disease Response Criteria

	Patient disease status at enrollment		
	MRD-positive disease < 5% blasts, morphologic, bone marrow AND > 0.01% blasts by flow cytometry, $\geq 10^{-4}$ by PCR, and/or $\geq 10^{-5}$ by NGS	**Low-Burden disease** $\geq$ 5% to < 25% blasts, morphologic, bone marrow	**High Burden Disease** $\geq$ 25% blasts, morphologic, bone marrow
Patient disease status after treatment with SJCAR19 < 5% blasts by morphologic evaluation of the bone marrow AND < 0.01% blasts by flow cytometry, $< 10^{-4}$ by PCR, and/or $< 10^{-5}$ by NGS	Complete Response, MRD-negative	Complete Response, MRD-negative	Complete Response, MRD-negative
< 5% blasts by morphologic evaluation of the bone marrow AND > 0.01% blasts by flow cytometry, $\geq 10^{-4}$ by PCR, and/or $\geq 10^{-5}$ by NGS	No Response	Complete Response, MRD-positive	Complete Response, MRD-positive
5% to 25% blasts by morphologic evaluation of the bone marrow, with confirmation by flow cytometry, PCR, and/or NGS	Progressive Disease	No Response	Partial Response
25% blasts by morphologic evaluation of the bone marrow, with confirmation by flow cytometry, PCR, and/or NGS	Progressive Disease	Progressive Disease	No Response
For participants who achieved remission after SJCAR19 infusion, the new finding of $\geq$ 5% leukemic blasts in the bone marrow, >0.01% blasts by flow cytometry $\geq 10^{-4}$ by PCR, $\geq 10^{-5}$ by NGS or the development of extramedullary disease	Relapse	Relapse	Relapse

8.0 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF-STUDY CRITERIA

8.1 Off Study Criteria

Recipient research participants will remain on study until one of the following occurs:

8.1.1 Withdrawal from protocol.

8.1.2 Death.

8.1.3 If patient eligible and consented to a portion of the protocol, but then is found to be ineligible for subsequent portions of the protocol.

8.1.4 Positive pregnancy test after enrolling in the protocol, but prior to receiving the study infusion.

8.1.5 Development of a significant change in health status at any point in therapy, which in the judgment of the clinical PI, would render continuation in the study medically unsafe or not in the participant's best interest

8.1.6 Unable to be contacted and/or effectively monitored by the Principal Investigator (PI) and/or designees for follow-up (lost to follow-up).

8.1.7 One-year after the last SJCAR19 infusion AND enrollment in the St. Jude long-term follow-up protocol for genetically modified cell therapy products.

8.1.8 Completion of long-term follow-up per FDA guidance.

8.2 Off Therapy Criteria

Recipient research participants may remain on study for monitoring of relapse, death and RCL, but be considered off therapy, if one of the following occurs:

8.2.1 Noncompliance with protocol medications/administrations and/or required follow-up evaluations.

8.2.2 Treatment on a therapeutic study or non-protocol treatment plan, or receipt of anti-leukemia therapy that is not specifically defined in this protocol, that would render the ongoing study of the SJCAR19 product or its toxicities no longer relevant (in the opinion of the study PIs). *Exception – need for bridging therapy for patients preparing for reinfusion of the SJCAR19 product.

8.2.3 Development of an unacceptable toxicity, which in the opinion of the clinical PI(s), would render continued participation harmful.

8.2.4 Receipt of any therapy during the initial 4 week evaluation period that could interfere with the SJCAR19 product *in vivo* (ex- chemotherapy, systemic steroids), except if that therapy is for the treatment of toxicity that is felt to be due to the SJCAR19 therapy.

9.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

9.1 Reporting Adverse Events and Deaths to St. Jude IRB

The principal investigator(s) is responsible for promptly reporting to the IRB any adverse events that are unexpected/unanticipated, serious, and that may represent potential harm or increased risk to research participants. When an unexpected death occurs, the PI should report it to the Director of Human Subject's Protection immediately, by phone: (901) 595-4359, cell: (901) 336-2894, fax: (901) 595-4361, or e-mail: hsp-1@stjude.org. A reportable event entry into iRIS should follow within 48 hours of notification of the event.

Serious, unexpected, and related, or possibly related, events must be reported within 10 business days of notification of the event. At the same time, the investigator will notify the FDA, as appropriate. All other SAEs, including expected death, and all captured AEs will be reported to the IRB at the time of the continuing reviews, with the exceptions of dose limiting toxicities defined in Table 5 (section 11.2).

For Phase I: Data on all adverse experiences/toxicities regardless of seriousness must be collected for documentation purposes.

For Phase II (initial and any reinfusions): Data on adverse events ≥ grade 3 will be recorded. In addition, clinically significant grade 1-2 adverse events that are judged to be related/possibly related to the CAR T-cell product may be collected per the discretion and judgment of the PI. Examples could include but are not limited to: events meeting criteria for SAE and infections requiring oral systemic therapy.

For both Phase I/II: In accordance with our institutional practice, data will be collected on related AEs for 7 days after the apheresis procedure. AE collection will then resume for the treatment portion of the study, beginning on the day of starting lymphodepleting chemotherapy and continuing for 4 weeks post-SJCAR19 infusion. Collection of AEs related to CRS and/or neurotoxicity will continue to be collected until the 3 month time point after the last SJCAR19 infusion. Reporting of serious adverse events related to gene transfer will occur initially on protocol for 1 year after the last SJCAR19 infusion, and then continue as part of our existing institutional long-term follow-up protocol or on SJCAR19 for patients who do not enroll on this LTFU study.

The criteria listed in the NCI Common Toxicity Criteria Scale will be used in grading toxicity (Version 5.0 located at http://ctep.cancer.gov) with the exception of CRS and

neurological toxicities that are related to SJCAR19. CRS and neurological toxicities will be graded according to Appendices G and H.

The following definitions apply with respect to reporting adverse experiences:

Serious adverse event – any adverse event temporally associated with the participant's participation in research that meets any of the following criteria:

- results in death;
- is life-threatening (places the participant at immediate risk of death from the event as it occurred);
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or
- any other adverse event that, based upon appropriate medical judgment, may jeopardize the participant's health and may require medical or surgical intervention to prevent one of the above outcomes.

Unexpected adverse event – any adverse event meeting any of the following criteria:

- an event for which the specificity or severity is not consistent with the protocol related documents, including the applicable investigator brochure, IRB approved consent form, IND application or any of the product labeling or package inserts;
- an event for which the observed rate of occurrence is significantly increased above what is expected or credible baseline rate for comparison;
- an event for which the occurrence is not consistent with the expected natural progression of any underlying disease, disorder, or condition of the participant(s) experiencing the adverse event and the participant's predisposing risk factor profile for the adverse event.

The principal investigator(s) is responsible for reviewing the aggregate toxicity reports and reporting to the IRB if the frequency or severity of serious toxicities exceed those expected as defined in the protocol or based on clinical experience or the published literature. Any proposed changes in the consent form or research procedures resulting from the report are to be prepared by the study team and submitted with the report to the IRB for approval.

9.2 Reporting to St. Jude Institutional Biosafety Committee

Continuing review reports will be sent to the Institutional Biosafety Committee (IBC) on an annual basis using the most current version of the continuing review form found on the IBC website. The safety reports, sent to the IRB for both the donors and recipients, will be simultaneously forwarded to the IBC. Therefore, reporting for safety events to this committee will be according to the same timelines as reporting to the IRB. This includes notification of achievement of MTD (if/when applicable). As per the direction of the IBC, only those protocol revisions and amendments directly related to the CliniMACS processing and related reagent(s) will require review and consideration by the IBC. Other revisions/amendments will be noted in the IBC continuing review report.

9.3 Reporting to FDA (21CFR§312.32 Safety reports)

The FDA will be notified in writing (IDE safety report) of any serious and unexpected AE associated with an investigational treatment or device; or any results from laboratory animal tests that suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.
Each notification to the FDA should be made as soon as possible and no later than 15 calendar days after the sponsor's initial receipt of the information. The FDA may require additional data to be submitted. In each written IND safety report, the sponsor shall identify all safety reports previously filed with the IND concerning a similar adverse experience, and shall analyze the significance of the adverse experience in light of the previous, similar reports where applicable.

The sponsor shall notify the FDA by telephone or by facsimile transmission of any death that occurs within 30 days after infusion of the SJCAR19 product; notification will occur as soon as possible, but no later than 7 calendar days after the sponsor's initial receipt of the information. Any grade III-IV infusion reactions will be reported as soon as possible, but every effort should be made to assure reporting is no more than within 7 business days of the event. In addition to all suspected unexpected serious adverse reactions, Grade 4 CRS and neurologic toxicities will be reported to the FDA in an expedited fashion. Follow-up information to a safety report must be submitted as soon as the relevant information is available.

If the results of further investigation show an AE that was not initially determined to be reportable should later be deemed reportable, the sponsor shall inform the FDA of the event in a written safety report as soon as possible, but no later than 15 calendar days after the determination is made. Results of the investigation of other safety information shall be submitted, as appropriate, in an information amendment or annual report. Continuing review reports, which will include the up-to-date clinical and safety data, will be submitted to the FDA at least annually.

9.4 Reporting to St. Jude Office of Regulatory Affairs

Copies of all correspondence to the St. Jude IRB, including SAE reports are provided to the St. Jude Regulatory Affairs Office. All FDA related correspondence and reporting will be conducted through the Regulatory Affairs Office. Adverse event reporting and annual reporting will be in accord with the FDA Title 21 CFR312.32 and Title 21 CFR312.33, respectively. The Regulatory Affairs Office can be reached at 901-595-2347 (secondary contact: St. Jude Vice President of Clinical Trials Administration 901-595-2876).

9.5 Continuing Review Reports

Continuing review reports of protocol progress and summaries of adverse events will be filed with the St. Jude IRB and IBC at least annually. Continuing review reports to all regulatory authorities will be structured in a manner so that any infusion toxicities or stem cell product related variances will be reported in separate listings from all other required elements.

9.6 Reporting to the Center for International Blood and Marrow Transplant Research

The Transplant Program at St. Jude is required by the federal government to report transplant information to the Center for International Blood and Marrow Transplant Research (CIBMTR). The CIBMTR is a research partnership of the International Bone Marrow Transplant Registry, the National Marrow Donor Program (NMDP), and the Foundation for the Accreditation of Cellular Therapy (FACT). This organization is responsible for the collection and maintenance of a standardized data warehouse registry of autologous and all allogeneic (related and unrelated donor) transplants and cellular infusions performed in the United States.

The Office of General Counsel, U.S. Department of Health and Human Services, had deemed the CIBMTR not a covered entity under the Privacy Rule (45 CFR 164.512), 45 CFR Parts 160 and 164, and the Health Insurance Portability and Accountability Act (HIPPA) of 1996. For this reason, the submission and disclosure of certain protected health information (PHI), including that required for CIBMTR, is allowable without the individual's authorization (i.e. consent is waived) when such disclosure is made to public health authorities authorized by law for the purpose of preventing or controlling disease, injury, or disability.

Data resulting from this procedure may be sent for general registry purposes to comply with the federal government requirements. This information is submitted using a unique participant identification number. The information submitted is less extensive than recipients of other donor products. For this reason, variables submitted may include but are not limited to the recipient's date of birth, country/state of current residence, diagnosis, basic lympho-hematopoietic reconstitution post-HCT disease status, and basic AEs, survival status, date/cause of death.

10.0 DATA COLLECTION, STUDY MONITORING, AND CONFIDENTIALITY

10.1 Data Collection and Submission

The Clinical Research Associates (CRAs) and CRA-RNs, within the Department of BMTCT will assure protocol compliance, and conduct all clinical and safety data collection. Data will be entered into the St. Jude institutional database at the time it is obtained from the electronic health record. The PI and/or designee will be responsible for the review of data for accuracy and completeness. The PI and/or designee will review the study data at least monthly.

10.2 Study Monitoring

This protocol will be monitored for safety and data as per the St. Jude Data and Safety Monitoring Plan for Clinical Trials approved by the NCI in 2010. The study team will meet at appropriate intervals to review case histories and data quality summaries on all participants.

The St. Jude Clinical Trials Office (CTO) Eligibility Coordinators will verify 100% of the informed consent documentation on all participants and verify 100% of participant's eligibility status within 5 working days of completion of enrollment.

The St. Jude CTO will track accrual continuously and verify 100% of all data points related to the primary and secondary objectives including AEs/SAEs every 6-8 weeks to assess overall study conduct, human subjects' protections, and the accuracy of database entries. Essential regulatory documents and all study documents including medical records, electronic media, database entries, study worksheets, and case report forms will be reviewed for recording and reporting of Adverse Events/Serious Adverse Events (SAEs) to include type, grade, attribution, duration, timeliness and appropriateness. Study documents are also reviewed for participant status, eligibility, the informed consent process, demographics, staging, study objectives, subgroup assignment, treatments, evaluations, responses, off-study and off-therapy criteria, and for all other specifics as detailed in the protocol. The Clinical Research Monitor will generate a formal report which is shared with the Principal Investigator, study team and the Internal Monitoring Committee (IMC).
Monitoring may be conducted more frequently if deemed necessary by the CTO or the IMC.

Continuing reviews by the IRB and CT-SRC will occur at least annually. In addition, SAE reports in iRIS are reviewed in a timely manner by the IRB/OHSP.

The Regulatory Affairs Office will assist the PI in reporting to the FDA and other external oversight agencies, as necessary.

10.3 Confidentiality

Unique patient numbers will be used in place of an identifier such as a medical record number. This unique patient number will be used to identify any data that is released to persons or agencies outside of the study team. No research participant names will be recorded on the data collection forms. The list containing the unique patient number and the medical record number will be maintained in a password protected file that is accessible only to study team members.

10.4 Genomic Data Sharing

Genomic and epigenomic data may be shared through the St. Jude Cloud and the Database for Genotypes and Phenotypes (dbGAP) and the Gene Expression Omnibus (GEO), which are both run by the NIH, and the Sequence Read Archive (SRA). Prior to submitting data, data will be stripped of identifiers such as name, date of birth, medical record number, and any other information that could be used to identify participants and will be fully de-identified by standards consistent with the Common Rule and HIPAA. The genotype data will be made publicly available no later than six months after completion of sequencing and analysis for all patients on the study, or the date of initial publication, whichever comes first.

11 STATISTICAL CONSIDERATIONS

11.1 Overview of Statistical Design and Analysis

Once a patient is eligible/consented to proceed with treatment with SJCAR19, there are two different phases of assessing whether SJCAR19 is safe and effective. The first phase of the study is to determine the maximum tolerated dose (MTD). The statistical design for this phase is a traditional 3 + 3 Phase I design. The second phase of the study will evaluate, using a Simon's two-stage minimax design, evidence that SJCAR19 would be expected to result in complete response (CR) rates in an acceptable proportion of treated patients with MTD at enrollment, as well as further inform on the safety of this therapy. The statistical design for the Phase II study is an expansion cohort at the MTD determined in the Phase I portion of the study. Those treated at the MTD in the Phase I study will be evaluated for efficacy as part of the Phase II study. In both the Phase I and II portions of the study, the primary objectives evaluation period is 4 weeks after initial infusion with SJCAR19. Long-term follow-up will occur for 15 years as per FDA guidance. Follow-up will occur on protocol for 1 year after the last SJCAR19 infusion. After one year, patients will be approached and consented to our existing long-term follow-up institutional protocol. If a patient does not enroll on this study, they will continue to be monitored on-study (SJCAR19) yearly, for up to 15 years post-infusion, per FDA guidance.

Only patients, who are enrolled on the treatment portion of the protocol (Phase I or Phase II [1st infusion only]), will be **replaced** for the following reasons:

- Lost for follow-up within the first 4 weeks post-SJCAR19 infusion (only for Phase I)
- Meet any of the off-study or off-therapy criteria listed below within the first 4 weeks post-SJCAR19 infusion:
  1) Noncompliance with protocol medications/administrations and/or required follow-up evaluations.
  2) Positive pregnancy test after enrolling in the protocol, but prior to receiving the study infusion.
  3) Enrollment onto a therapeutic study or non-protocol treatment plan, or receipt of anti-leuekmia therapy that is not specifically defined in this protocol, that would render the ongoing study of the SJCAR19 product or its toxicities no longer relevant (in the opinion of the study PIs) (only for Phase I).
  4) Receipt of any therapy during the initial 4 week evaluation period that could interfere with the SJCAR19 product *in vivo* (ex- chemotherapy, systemic steroids), except if that therapy is for the treatment of toxicity that is felt to be due to the SJCAR19 therapy (only for Phase I).
  5) Withdrawal from protocol.
  6) If patient eligible and consented to a portion of the protocol, but then is found to be ineligible for subsequent portions of the protocol.

7) Development of a significant change in health status at any point in therapy, which in the judgment of the clinical PI, would render continuation in the study medically unsafe or not in the participant's best interest.

- Death due to progressive disease within the first 4 weeks post-SJCAR19 infusion (only for Phase I)

- Do not receive all of protocol mandated therapy such as:
  - If the patient is unable to receive the protocol defined lymphodepleting chemotherapy regimen of fludarabine/cyclophosphamide. They may still receive the SJCAR19 infusion, after no chemotherapy or an alternative chemotherapy regimen. These patients will be followed per protocol, but will be replaced.

With this study plan, the minimum number of patients (whom received protocol defined dosing of SJCAR19 and are evaluable at the 4 week time point) needed for the Phase I portion of the study would be 9: 3 on dose level 1 and 6 on dose level -1. The maximum number of patients (whom received protocol defined dosing of SJCAR19 and are evaluable at the 4 week time point) for Phase I would be 12: 6 patients on each dose level 1-2. The Phase II expansion cohort will include 12 additional patients (whom received protocol defined dosing of SJCAR19 and are evaluable at the 4 week time point) treated at the MTD determined in the Phase I trial. So the total number of patients for the study would be minimum of 21 and maximum of 24. Because the patients who are unable to receive the fludarabine/cyclophosphamide lymphodepleting regimen or die due to progressive disease will be replaced, we will continue to enroll patients on the study to ensure we will have the sufficient number of evaluable patients to assess the primary safety and efficacy endpoints.
Based on our St. Jude protocol NKHEM, the total 24 patients were enrolled on the study in five years. With an estimated accrual rate of about 6 participants per year, the study is expected to last for about 5-6 years.

11.2 Dose Limiting Toxicity

A dose limiting toxicity is defined as any toxicity that is considered to be at least possibly related to SJCAR19, which is irreversible or life-threatening.

DLTs will be monitored for 4 weeks post-infusion of SJCAR19, in both the phase I and II portions of the study. DLTs to be monitored are shown in Table 5. All toxicities, with the exception of cytokine release syndrome (CRS) and neurotoxicity, will be determined and graded using the NIH/NCI Common Terminology Criteria for Adverse Events (CTCAE v5.0). Abnormal metabolic values will be captured but not considered a DLT unless they meet DLT grading limits and the criteria of SAE or are deemed as clinically significant per the judgment of the PI. CRS and neurotoxicity will be assessed and graded using the ASTCT consensus grading statement for CRS and Neurotoxicity[58] (Appendices G and H). Any toxicity that is felt to be resulting from CRS or neurotoxicity will be attributed to CRS or neurotoxicity (ex. fever,

oxygen requirement, hypotension, aphasia, confusion, renal insufficiency, transaminases[57, 58, 62]), and not be considered a DLT unless they meet DLT grading limits and the criteria of SAE or are deemed as clinically significant per the judgment of the PI.

In both the Phase I and II portions of the study, in the event that a DLT is observed, the treatment phase of the study will be paused and the DLT will be discussed with the study team and the appropriate regulatory committees and agencies including the FDA. Based on this review, the study may resume as written, or with modifications.

Table 5: Dose-Limiting Toxicities

Treatment-related death (Grade 5)
Grade 4 hematologic toxicity that fails to return to Grade 3 or baseline (whichever is more severe) within 4 weeks, except for lymphopenia
Grade 3 to 4 allergic reaction to SJCAR19 infusion
Grade 3 to 4 treatment-emergent autoimmune toxicity
Grade 3 CRS or neurotoxicity (protocol defined) that fails to resolve to ≤ Grade 2 within 7 days of onset[#]
Grade 4 CRS or neurotoxicity (protocol defined) that fails to resolve to ≤ Grade 3 within 72 hours of onset[#]
In addition to those criteria listed above: A dose limiting toxicity is defined as any non-hematologic toxicity* that is considered to be at least possibly related to the SJCAR19 product, which is irreversible** or life-threatening***, and is not pre-existing, due to underlying malignancy and/or associated with CRS/Neurotoxicity. *graded using CTCAE v5.0, with exception of CRS/Neurotoxicity (protocol defined). Abnormal metabolic values will be captured but not considered DLTs unless they meet the criteria of SAE or deemed as clinically significant per the judgment of the PI. **A toxicity will be considered irreversible if it does not resolve to ≤ Grade 2 or baseline (whichever is more severe) by day 28 post-infusion of the SJCAR19 product. ***A toxicity will be considered life-threatening if it places the participant at immediate risk of death from the event as it occurred, per the judgement of the PI.

#The following Grade 3 events, with or without association with CRS and/or Neurotoxicity, will be considered DLTs if they fail to resolve to ≤ Grade 2 or baseline (whichever is more severe) within 72 hours of onset
- Nervous System disorders:
  o Amnesia
  o Aphonia

- Arachnoiditis
- Brachial plexopathy
- Central Nervous System Necrosis
- Cerebrospinal fluid leakage
- Depressed level of consciousness
- Intracranial hemorrhage
- Myelitis
- Pyramidal tract syndrome
- Radiculitis
- Recurrent laryngeal nerve palsy
- Seizure (multiple seizures)
- Somnolence
- Stroke
- Transient ischemic attack

- Cardiac disorders:
  - Aortic Valve disease
  - Atrial fibrillation
  - Atrial flutter
  - AV block complete
  - Chest pain
  - Conduction disorder
  - Mitral valve disease
  - Mobitz type II and type I
  - Myocardial infarction
  - Myocarditis
  - Pericarditis
  - Pulmonary valve disease
  - Restrictive cardiomyopathy
  - Sick sinus syndrome
  - Sinus bradycardia
  - Tricuspid valve disease
  - Ventricular arrhythmia
  - Congenital, familial and genetic disorders- other, specify

11.3 Dose Escalation

A cohort of 3 participants is treated at a particular dose level and if 0/3 participants experience a dose-limiting toxicity (DLT) as defined in Table 5 in the toxicity evaluation period, then the next cohort of 3 is treated at the next higher dose level. If 1/3 experiences DLT then an additional cohort of 3 participants is treated at the same dose level. If 1/6 participants experiences DLT at this level then the next cohort of 3 participants will be treated at the higher dose level. If 2+/6 (or 2+/3 in the initial cohort) experience DLT, then that would mean that the MTD has been exceeded. In such a situation, the previous dose level is declared to be the MTD if 6 participants have already been treated at that dose level, otherwise an additional cohort of 3 participants is treated at that dose level. The MTD is then defined as the highest dose level at which 6 participants have been treated with no more than 1 instance of DLT. Finally, if there is no DLT on the last dose level 2, we will claim MTD not reached. If there is 2 or more DLT in the dose level 1, then dose level 0 will be evaluated. The

dose escalation schema and toxicities observed and the actions to be taken are listed below (Table 6).

In the Phase I study, patients will be enrolled on a particular treatment dose level on a staggering basis. For each dose level being evaluated, there will be a 4 week period between SJCAR19 infusions for patients 1 and 2. This time period should allow for sufficient length of time for the monitoring of adverse events, as the most common anticipated AE is CRS and it has been reported that the median time to onset of CRS is 3 – 4 days. However, at no time the number of participants experienced DLT within 4 weeks plus the number of participants receiving the SJCAR19 infusion and not yet passing 4 weeks' evaluation will exceed two (2). Additionally, the first three (3) patients treated on study must be aged ≥ 16 years old.

Table 6: **The toxicities observed and the actions to be taken**

# Patients	# DLTs	Action
3	0	escalate to next level
3	1	enroll 3 more at this level
6	1	escalate to next level
3-6	2	declare MTD exceeded and treat 3 more at the previous dose level if only 3 were treated; or the previous dose is MTD if 6 were treated at the previous dose

11.4 Efficacy and Stopping Rules

The complete response rate for standard therapy is 40%. The goal of our study is to use SJCAR19 to improve the treatment outcome at a complete response (CR) rate of ≥70%. We do not anticipate censoring within 4 weeks after SJCAR19 infusion and we can approximate the rate of CR within 4 weeks after SJCAR19 infusion using a Binomial distribution. (However, within 4 weeks, patients lost for follow up, who die due to treatment related toxicity, enroll onto a therapeutic study or non-protocol treatment plan, or receipt of anti-leuekmia therapy that is not specifically defined in this protocol, that would render the ongoing study of the SJCAR19 product or its toxicities no longer relevant (in the opinion of the study PIs), or receive any therapy that could interfere with the SJCAR19 product *in vivo* (ex-chemotherapy, systemic steroids), except if that therapy is for the treatment of toxicity that is felt to be due to the SJCAR19 therapy will be counted as a failure to complete response in order to keep the validity of Binomial distribution approximation). Therefore, in this study, we propose to test the null hypothesis H_0: $P \leq 0.40$ versus H_1: $P > 0.40$, where P is the proportion of research participants with complete response within 4 weeks after the SJCAR19 infusion. With type I error of 5% and type II error of 20%, Simon's two stage minimax design powered at alternative successful complete response rate $P_1=0.70$ requires 12 evaluable patients at the first stage and 18 evaluable patients in total.[87] The stopping rules are provided in Table 7, with the understanding that stopping the trial early would be suggestive of the proposed new treatment strategy not being an effective treatment option for this group of patients.

The interpretation is that if we observe ≤ 6 participants with complete response within 4 weeks after SJCAR19 infusion in 12 participants treated at the MTD (6 treated at MTD in Phase I, 6 enrolled at MTD on Phase II), then we would stop the trial for lack of efficacy and will not enroll another 6 patients. The probability of stopping the trial early if the true CR rate is 0.4 or less, is 0.84. However, if we observe ≥ 7 patients with complete response within 4 weeks after SJCAR19 infusion in the initial 12 participants treated at the MTD, then 6 more patients will be enrolled on the Phase II portion of the study.

After the study is finished, the function *twostage.inference* in clinfun R package that accounts for the two-stage design will be used to calculate the confidence interval.

Table 7: Stopping rules for lack of efficacy based on the Simon's 2-stage minimax design (unacceptable low rate of complete response within 4 weeks after the T cell infusion)

Accept H_0 if the number of research participants with complete response					
P_0	P_1	($\leq r_1/n_1$)	($\leq r/n$)	EN(P_0)	PET(P_0)
0.40	0.70	6/12	10/18	12.9	0.84

Note: r_1 and r denote the number of patients with complete response within one month after the completion of therapy; EN(P_0) denotes the expected sample size under P_0; PET(P_0) denotes the probability of early termination at stage I under P_0.

Additionally, we will pause treatment study enrollment, pending further investigation, for any Grade 5 AE other than that related to progressive disease, or if 2 patients experience a Grade 4 AE related to vital organs (that is not included in the CRS/neurotoxicity grading system), that occurs within 4 weeks of infusion of SJCAR19.

Data from any reinfusions may be included in safety, efficacy and exploratory analysis, but will not be included in primary objective analysis. This data will be collected as a unique infusion, but not as a unique patient (i.e. reinfusion does not count towards enrollment goals).

11.5 Analysis for Exploratory Objectives

Responsible investigators: All investigators will be responsible for all exploratory objectives except 11.5.10-11.5.11.

11.5.1 To describe the feasibility of manufacturing SJCAR19 for pediatric and young adult patients ≤ 21 years of age, with relapsed or refractory $CD19^+$ ALL, and explore possible factors contributing to manufacturing failure.

The number of patients who do not receive the protocol defined dose of SJCAR19 will be reported. To explore possible factors contributing to manufacturing failure, we will compare factors between patients who do and who don't receive the protocol defined dose of SJCAR19. Depending

on the number, summary statistics including the number and frequency for categorical variables and mean, SD, median and range for continuous variables will be reported. They will be compared using Fisher's exact test for categorical variables and two-sample t-test or Wilcoxon rank sum test depending on the normality of the data tested with Shapiro-wilk test for continuous variable, respectively. Generalized linear regression model may also be used to explore possible factors contributing to the manufacturing failure.

Time frame: The final analyses will begin 1 day after the last patient is enrolled and treated.

11.5.2 To evaluate the relapse-free survival of patients with minimal residual disease at the time of treatment with SJCAR19.

The Kaplan-Meier estimates of relapse-free survival of patients with minimal residual disease along with their standard errors will be calculated using the SAS macro (bmacro251-Excel2007\kme) available in the Department of Biostatistics at St. Jude, where EFS = *min* (date of last follow-up, date of relapse) – date of treatment, and all participants surviving at the time of analysis without events will be censored. The analysis for this objective will be performed when the last evaluable participant has been followed for one-year post treatment. Because patients with minimal residual disease dying from toxicity will be competing risk event for relapse, CI analyses will also be conducted.

Time frame: The final analyses will begin 1 year after the last patient is enrolled and treated.

11.5.3 To study the expansion, persistence and phenotype of SJCAR19.

These samples will be collected over a year. Variables will be expansion (number of detectable SJCAR19 cells), persistence (presence of detectable SJCAR19 cells) and phenotype (cell surface markers of SJCAR19 cells). At each time point, summary statistics including mean, SD, median and range will be reported. Box-cox plot will also be used to visually look at the trend of the expansion, persistence and phenotype of SJCAR19 over time. The change over time for the expansion, persistence and phenotype of SJCAR19 will be estimated using linear mixed model with assessment times as a covariate. We will also explore if there is difference between patients in phase I and those on MTD in phase II by including group as a covariate in the model.

Time frame: The final analyses will begin 1 year after the last patient is enrolled and treated.

11.5.4 To characterize the cytokine profile in the peripheral blood and CSF after treatment with SJCAR19.

These samples will be collected over a year. Variables will be levels of specific cytokines. The analyses methods will be similar to those for objective 11.5.6.

Time frame: The final analyses will begin 1 year after the last patient is enrolled and treated.

11.5.5 To explore the use of next-generation sequencing (NGS) for the monitoring of disease status post-treatment with SJCAR19 compared to minimal residual disease detection via flow cytometry.

Since NGS is more sensitive to monitor disease post treatment with SJCAR19, the sensitivity and specificity comparison between NGS and the flow cytometry will be performed for patients for whom both assays are performed. The agreement in percent of leukemia cells using NGS and flow cytometry will also be evaluated using intraclass correlation coefficient.

Time frame: The final analyses will begin 1 year after the last patient is enrolled and treated.

11.5.6 To determine the immune reconstitution post SJCAR19 treatment, and clonal structure and endogenous repertoire of CAR T cells during in vitro and in vivo expansion.

The standard protocols for repertoire analysis and quantitative assessment of repertoire diversity and specificity will be used on longitudinal samples from in vitro and in vivo expanded CAR T cell populations. Outcomes will include the diversity (assessing both richness and evenness) of CAR T cell and unmodified immune cell populations over time and the identification of receptor families from known specificities (e.g. common infections such as influenza, herpesvirus etc) that preferentially associate with expanded clones. VIS analysis will also be performed.

Time frame: The final analyses will begin 1 year after the last patient is enrolled and treated.

11.5.7 To assess whether CAR T cells acquire functional versus exhaustion-associated epigenetic programs during in vitro and in vivo expansion.

Loci-specific analyses can be performed with a few hundred cells, and we have currently optimized conditions to perform whole-genome bisulfite sequencing on ~20,000 cells. Loci-specific analyses may include regions of the genome that code for effector molecule, inhibitory receptor, and homing molecule loci (e.g. granzyme b, perforin, PD-1, CTLA4 Lag3, 2B4, and CD62L). We will perform loci-specific analyses with samples that yield <10,000 PD-1+ T cells. Whole genome bisulfite sequencing may be performed on samples that yield >10,000 PD-1+ T cells.

Time frame: The final analyses will begin 1 year after the last patient is enrolled and treated.

11.5.8 To longitudinally assess and quantify the symptoms, associated distress, and functional impairment experienced by patients enrolled on this Phase I/II clinical trial.

The scores of each PRO-CTCAE attribute (frequency, severity, and/or interference) at all time points will be summarized by descriptive statistics separately for the patient and parent proxy groups, e.g., mean, SD, median and range, proportion. If an adequate sample size is available in each group, the changes of scores at each pair of adjacent time points will be tested with Wilcoxon signed-rank test.

Time frame: The final analyses will begin 1 year after the last patient is enrolled and treated.

11.5.9 To longitudinally assess and quantify numerous metrics of quality of life and well-being for patients enrolled on this Phase I/II trial and their primary caretakers.

11.5.9.1

The total and subscale scores of PedsQL V. 4 and PedsQL – Cancer V. 3 at all time points will be summarized by descriptive statistics separately for the patient and parent proxy group. The scores measuring the quality of life and well-being for the caretaker at all time points will also be summarized by descriptive statistics. The correlations among the scores will be estimated by Pearson correlation coefficient or Spearman's rank correlation coefficient and tested by Fisher transformation. The scores between the groups, e.g., high and low burden, will be tested with Wilcoxon rank-sum test.

The changes of scores at each pair of adjacent time points will be tested with Wilcoxon signed-rank test. If an adequate sample size is available, the patterns of the longitudinal change will be determined and linear mixed effects or GEE model will be fitted to the scores to validate the longitudinal trends and the relations among the scores.

Time frame: The final analyses will begin 1 year after the last patient is enrolled and treated.

11.5.9.2

Semi-structured interviews will be recorded and manually transcribed into MaxQDA. Analysis of this data will be performed by trained members of the quality of life division using an applied thematic analysis approach, which is an iterative process of

identifying themes or units of meaning observed in the data. Members of the quality of life division with extensive experience in thematic analysis will jointly develop themes for all interviews, which they will then refine into codes with well-developed definitions. Once code definitions have been finalized, two additional individuals will serve as independent raters to evaluate the transcripts. These individuals will complete study specific training in content analysis and use of the codes. After completing training, the two independent raters will analyze the interviews. Kappa coefficients will be calculated to measure the inter-coder reliability between each of the raters.

Time frame: Qualitative analysis will be ongoing, with interviews being transcribed and analyzed as they are completed.

11.5.10 To characterize incidence and mechanisms of resistance and/or relapse post therapy with SJCAR19.

The estimate of cumulative incidence of relapse will be determined using Kalbfleisch-Prentice method. Death without relapse is the competing risk event. The Kaplan-Meier estimates of OS and EFS along with their standard errors will be calculated using the SAS macro (bmacro251-Excel2007\kme) available in the Department of Biostatistics at St. Jude, where OS = min (date of last follow-up, date of death) – date of infusion and all participants surviving at the time of analysis without events will be censored, and EFS = *min* (date of last follow-up, date of relapse, date of death due to any cause) – date of infusion, and all participants surviving at the time of analysis without events will be censored. The final analysis for this objective will be performed when the last evaluable participant has been followed for one-year post-CAR T-cell infusion.

12.0 OBTAINING INFORMED CONSENT

12.1 Informed Consent

This protocol contains a 3-part informed consent process. Patients that meet eligibility for autologous apheresis will be consented to undergo collection of autologous peripheral blood mononuclear cells (PBMC) via apheresis. Alternatively, patients whom have previously undergone autologous apheresis may be considered for enrollment on the manufacturing portion of the study if it is determined that their previously collected product is appropriate for use in manufacturing; these patients would therefore not need to be consented using the SJCAR19 apheresis consent. When the patient meets eligibility criteria to begin manufacturing of SJCAR19, they will be consented to proceed with manufacturing of the SJCAR19 product using their collected autologous cells. At this time, patients/guardians will be provided with an FYI consent for the treatment portion of the study. Lastly, when a SJCAR19 product has manufactured for an individual patient and they meet eligibility criteria to receive

therapy with SJCAR19, they will be consented to proceed with the treatment portion of the study.

12.2 Informed Consent Prior to Research Interventions

The PI or physician sub-investigator will conduct the signature authorization portion of the consent process. Authorization for the recipient procedure should be conducted in the presence of an independent witness, such as a nurse from the St. Jude Department of Nursing or the St. Jude Institutional Review Board Ombudsperson/Patient Advocate, as applicable and available to serve as a witness.

12.3 Consent at Age of Majority

The age of majority in the state of Tennessee is 18 years old. Research participants must be consented at the next clinic visit after the 18th birthday. If an affiliate is located in a country or state where a different age of majority applies, the location must consent the participants according to their local laws.

12.4 Consent When English is Not the Primary Language

When English is not the patient, parent, or legally authorized representative's primary language, the Social Work department will determine the need for an interpreter. This information documented in the participant's medical record. Either a certified interpreter or the telephone interpreter's service will be used to translate the consent information. The process for obtaining an interpreter and for the appropriate use of an interpreter is outlined on the Interpreter Services, OHSP, and CTO websites.

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APPENDIX A: Karnofsky and Lansky Performance Status

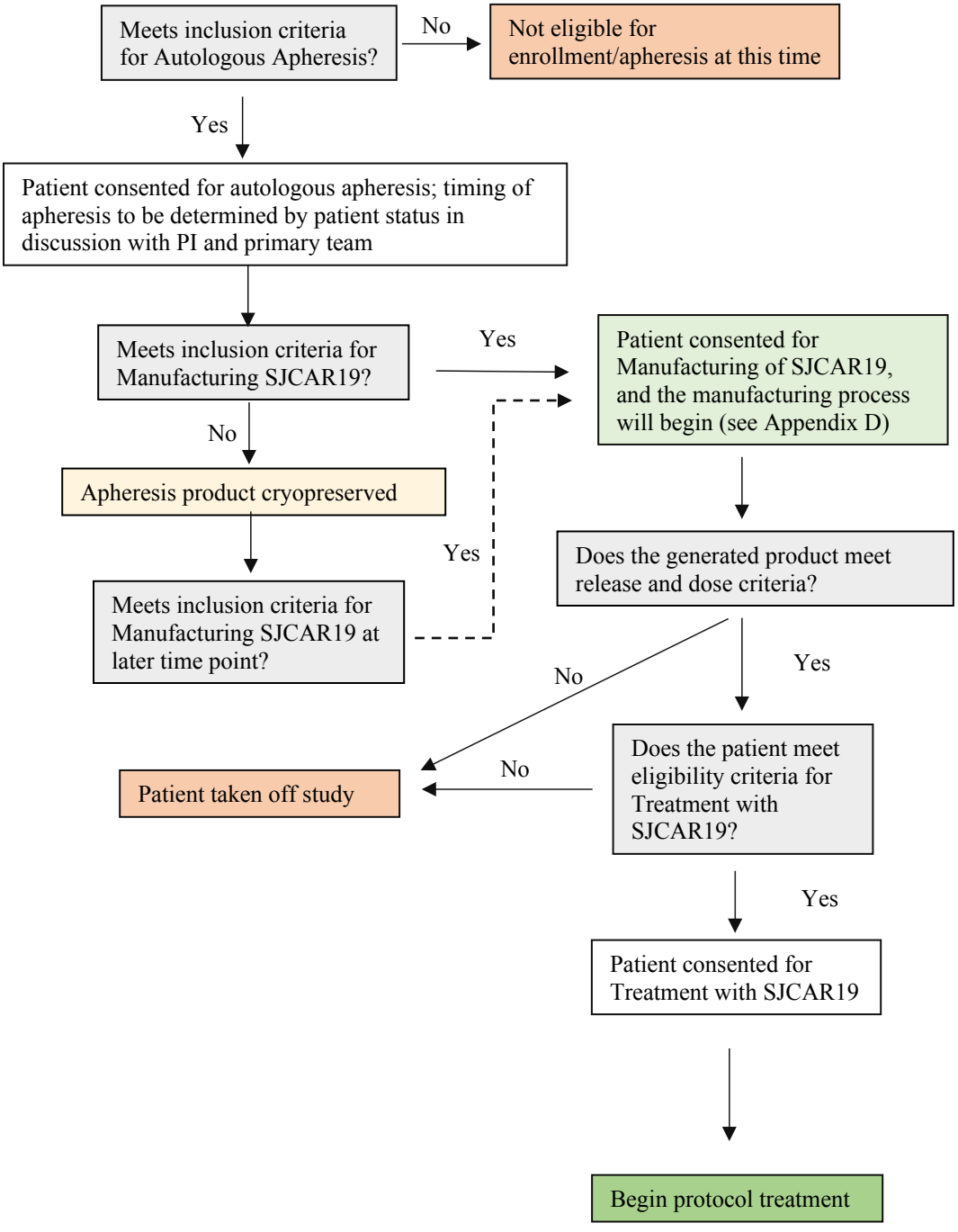
KARNOFSKY PERFORMANCE STATUS SCALE ≥ 16 YEARS OLD	
Score	**General Description**
100	Normal. No complaints. No evidence of disease.
90	Able to carry on normal activity. Minor signs or symptoms of disease.
80	Normal activity with effort. Some signs or symptoms of disease.
70	Care of self. Unable to carry out normal activity or to do active work.
60	Requires occasional assistance, but is able to care for most of his needs.
50	Requires considerable assistance and frequent medical care.
40	Disabled. Requires special care and assistance.
30	Severely disabled. Hospitalization is indicated although death is not imminent.
20	Hospitalization necessary, very sick, active support treatment necessary.
10	Moribund. Fatal processes progressing rapidly.
0	Dead.

LANSKY PERFORMANCE STATUS SCALE < 16 YEARS OLD	
Score	**General Description**
100	Fully active, normal
90	Minor restrictions in physically strenuous activity
80	Active, but tires more quickly
70	Both greater restriction of and less time spent in play activity
60	Up and around, but minimal active play; keeps busy with quieter activities
50	Gets dressed but lies around much of the day, no active play but able to participate in all quiet play and activities
40	Mostly in bed; participates in quiet activities
30	In bed; needs assistance even for quiet play
20	Often sleeping; play entirely limited to very passive activities
10	No play; does not get out of bed
0	Unresponsive

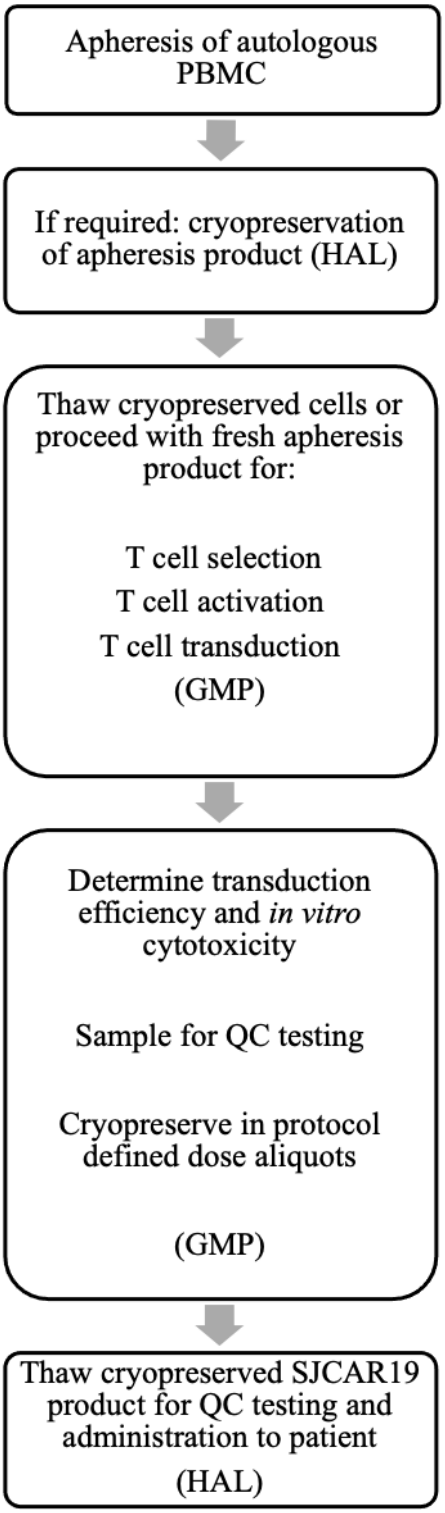
APPENDIX B: Steroid Equivalent Dose Reference Chart

	Standard Equivalent Dose	Protocol Equivalent Dose
Cortisone	25	3.125 mg/kg/day
Dexamethasone	0.75	0.09375 mg/kg/day
Hydrocortisone	20	2.5 mg/kg/day
Methylprednisolone	4	0.5 mg/kg/day
Prednisolone	5	0.625 mg/kg/day
Prednisone	5	0.625 mg/kg/day

APPENDIX C: Study Overview



APPENDIX D: Overview of SJCAR19 Manufacturing Procedure



APPENDIX E: Recommended Treatment Evaluations Study Calendar

Study Evaluation Target Windows				± 4 days			± 15 days		± 28 days		
Study	**Wk -1**	**Wk 0**	**Wk 1**	**Wk 2**	**Wk 3**	**Wk 4**	**Wk 8**	**Mth 3**	**Mth 6**	**Mth 9**	**Mth 12**
Cy + Flu^	X										
SJCAR19 Infusion		X									
Hx	X*	X**	X	X	X	X	X	X	X	X	X
PE	X*	X**	X	X	X	X	X				
Performance Status	X*	X**	X	X	X	X	X				
CBC with diff	X*	X**	X	X	X	X	X	X	X	X	X
Lytes, BUN, Cr	X*	X**	X	X	X	X	X				
AST/ALT, Bili, Alb	X*	X**	X	X	X	X	X				
CRP, Ferrtin	X*	X**	X	X	X	X	X				
Lymphocyte Subset Panel	X*					X	X	X	X	X	X
Quantitative Immunoglobulins	X*					X	X	X	X	X	X
Pregnancy Test#	X*										
RCL Testing	X*							X	X		X&
HAMA		X**				X					
ECHO	X@										
EKG	X@	X**									
Peripheral MRD	X%	X**				X		X			
BMA	X%					X		X			
CSF	X%					X		X†			
SJCAR19 Research Labs											
Cytokines	X*	X***	X	X	X	X	X				
Persistence Studies		X***	X	X	X	X	X	X	X	X	X
Function Studies	X*	X**	X	X	X	X	X	X	X	X	X
Leukemia/ Microenvironment[a]	X%					X					
For SJCAR19 Research Labs, additional samples may be sent if the patient has concern for disease relapse/progression, or an acute clinical change that is felt to possibly be related to the SJCAR19 product. Research laboratory testing may also be performed using a leftover clinically indicated sample.											

@ to be performed within 2 weeks prior to the start of protocol lymphodepleting chemotherapy

% to be performed after any bridging chemotherapy and within 2 weeks prior to start of protocol lymphodepleting chemotherapy

^ starts day -4, if unable to receive Cy or Flu, select patients may receive alternative regimen in discussion with PI prior to start of lymphodepleting chemotherapy

** post-lymphodepleting chemotherapy (if applicable)/pre T-cell infusion

*** pre T-cell infusion and 1 to 4 hours post infusion and an optional sample 3-4 days post infusion (optional per discretion of PI/Transplant Attending)

Pregnancy testing is only required in female patients of childbearing potential

† only for patients with recent history of CNS disease

& If a patient does not enroll on our institutional long-term follow-up protocol, we will continue to monitor RCL testing on-study per FDA guidance

a If a patient has circulating peripheral blood blasts at any time while on study, this analysis may also be perfomed on that study sample.

- Several laboratory tests can only be processed on weekdays; therefore, if the scheduled evaluation falls on a weekend, or during a holiday period, an adjustment in the follow-up visit is expected and would not be noted as a protocol variation. Additionally, in order to accommodate such logistical constraints, evaluation/collection dates of all protocol assessments (required and optional research), may be performed within a reasonable window of the intended date following the guidelines provided in the table headers.
- After week +4 participants may no longer be receiving treatment at St. Jude Children's Research Hospital or its affiliates. For this reason, variations in the timing and frequency (more or less frequent) of these evaluations, with the exception of the SJCAR19 RCL, and SJCAR19 Research labs, can occur due to the participant's clinical condition and will not be noted as protocol deviations after week +4.
- For any reinfusions, patients will follow this study calendar beginning with week -1

APPENDIX F: Criteria for Adverse Event (AE) Evaluation and Reporting

The St. Jude Department of BMTCT Clinical Research Office standard operating procedure for the documenting and reporting of adverse (SOP 10 Documenting and Reporting of Adverse Events https://home.stjude.org/bmt/Policies/10.pdf) will provide guidance on the evaluation, collection and reporting of adverse events for this clinical trial. The current version of this document, as well as ongoing updates, can be located at the following website: http://home.stjude.org/bmt/Pages/policies-research.aspx

APPENDIX G: Criteria for Cytokine Release Syndrome (CRS) Adverse Event (AE) Grading (ASTCT CRS Consensus Grading)

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

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

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

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

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

Symptoms should be graded individually using the CTCAE grading system, but will collectively be attributed/reported as part of the overall CRS grade**.** Symptoms and signs of CRS occurring after infusion of CAR T-cell products may include constitutional symptoms (fever, rigors, headache, malaise, fatigue, nausea, vomiting, arthralgia), vascular (hypotension), cardiac (arrhythmia, tachycardia), respiratory compromise, renal (rise in creatinine, kidney failure, uremia) and laboratory (including coagulopathy and a hemophagocytic lymphohistiocytosis-like syndrome) changes.

APPENDIX H: Criteria for Neurotoxicity Adverse Event (AE) Grading (ASTCT Neurotoxicity Consensus Grading)

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE score for children age ≥12 years*	7-9	3-6	0-2	0 (patient is unarousable and unable to perform ICE)
CAPD score for children age <12 years	1-8	1-8	≥9	Unable to perform CAPD
Depressed level of consciousness†	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Unarousable or requires vigorous or repetitive tactile stimuli to arouse; stupor or coma
Seizure (any age)	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (>5 min); or Repetitive clinical or electrical seizures without return to baseline in between
Motor weakness (any age)‡	N/A	N/A	N/A	Deep focal motor weakness, such as hemiparesis or paraparesis
Elevated ICP/ cerebral edema (any age)	N/A	N/A	Focal/local edema on neuroimaging§	Decerebrate or decorticate posturing, cranial nerve VI palsy, papilledema, Cushing's triad, or signs of diffuse cerebral edema on neuroimaging

ICANS grade is determined by the most severe event (ICE or CAPD score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause. Baseline CAPD score should be considered before attributing to ICANS.

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

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

‡ Tremors and myoclonus associated with immune effector cell therapies may be graded according to CTCAE v5.0, but they do not influence ICANS grading.

§ Intracranial hemorrhage with or without associated edema is not considered a neurotoxicity feature and is excluded from ICANS grading. It may be graded according to CTCAE v5.0.

Symptoms should be graded individually using the CTCAE grading system, but will collectively be attributed/reported as part of the overall neurologic toxicity grade; symptom examples are not all inclusive. examples include: mental status changes, tremor, agitation, aphasia, delirium, dizziness, dyskinesia, hallucination and restlessness

Appendix H (continued)

Encephalopathy Assessment Tool for Grading ICANS

ICE: (ages ≥ 12 years old)

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

CAPD: (< 12 years old)

Encephalopathy Assessment for Children Age <12 Years Using the CAPD

Answer the following based on interactions with the child over the course of the shift					
	Never, 4	Rarely, 3	Sometimes, 2	Often, 1	Always, 0
1. Does the child make eye contact with the caregiver?					
2. Are the child's actions purposeful?					
3. Is the child aware of his/her surroundings?					
4. Does the child communicate needs and wants?					
	Never, 0	Rarely, 1	Sometimes, 2	Often, 3	Always, 4
5. Is the child restless?					
6. Is the child inconsolable?					
7. Is the child underactive; very little movement while awake?					
8. Does it take the child a long time to respond to interactions?					

(Adapted from Traube et al [36]; reproduced with permission.)
For patients age 1-2 years, the following serve as guidelines to the corresponding questions:
1. Holds gaze, prefers primary parent, looks at speaker.
2. Reaches and manipulates objects, tries to change position, if mobile may try to get up.
3. Prefers primary parent, upset when separated from preferred caregivers. Comforted by familiar objects (ie, blanket or stuffed animal).
4. Uses single words or signs.
5. No sustained calm state.
6. Not soothed by usual comforting actions, eg, singing, holding, talking, and reading.
7. Little if any play, efforts to sit up, pull up, and if mobile crawl or walk around.
8. Not following simple directions. If verbal, not engaging in simple dialog with words or jargon.

APPENDIX I: Quality of Life Evaluations Study Calendar

QoL Related Studies Calendar* and Assessment Administration by Age and Time Point

- *QOL assessments may be conducted up to an additional 3 times during the first 4 weeks after cellular infusion as needed to capture peak symptoms; For any reinfusions, patients will follow this study calendar beginning with week 0

Study	Wk 0	Wk 2	Wk 4	Wk 8	Mth 3
PRO-CTCAE (English/Spanish)	X	X	X	X	X
PedsQL V.4 (English/Spanish)	X	X	X	X	X
PedsQL – Cancer V.3 (English/Spanish)	X	X	X	X	X
Good Day Bad Day Scale (English)	X	X	X	X	X
Qualitative Symptom Assessment (English)	X	X	X	X	X
CQOLC (English/Spanish)	X		X	X	X
FACIT-Sp-12 (English/Spanish)	X		X	X	X
Hospital Anxiety and Depression Scale (English)	X		X	X	X
Herth Hope Index (English/Spanish)	X		X	X	X
Modified Parent-Patient Activation Measure (English)	X		X	X	X

Assessment Administration by Age and Time Point

	Wk 0	Wk 2	Wk 4	Wk 8	Mth 3
Patients: 0-4 yrs	Caregiver Participation Only	Caregiver Participation Only	Caregiver Participation Only	Caregiver Participation Only	Caregiver Participation Only
Patients: 5-7 yrs	Good/Bad Day* Qual Symp* PedsQL PedsQL-C	Good/Bad Day* Qual Symp* PedsQL PedsQL-C	Good/Bad Day* Qual Symp* PedsQL PedsQL-C	Good/Bad Day* Qual Symp* PedsQL PedsQL-C	Good/Bad Day* Qual Symp* PedsQL PedsQL-C
Patients: 8-15 yrs	Good/Bad Day* Qual Symp* PedsQL PedsQL-C PRO-CTCAE (P)	Good/Bad Day* Qual Symp* PedsQL PedsQL-C PRO-CTCAE (P)	Good/Bad Day* Qual Symp* PedsQL PedsQL-C PRO-CTCAE (P)	Good/Bad Day* Qual Symp* PedsQL PedsQL-C PRO-CTCAE (P)	Good/Bad Day* Qual Symp* PedsQL PedsQL-C PRO-CTCAE (P)
Patients: 16-21 yrs	Good/Bad Day* Qual Symp* PedsQL PedsQL-C PRO-CTCAE (A)	Good/Bad Day* Qual Symp* PedsQL PedsQL-C PRO-CTCAE (A)	Good/Bad Day* Qual Symp* PedsQL PedsQL-C PRO-CTCAE (A)	Good/Bad Day* Qual Symp* PedsQL PedsQL-C PRO-CTCAE (A)	Good/Bad Day* Qual Symp* PedsQL PedsQL-C PRO-CTCAE (A)
Caregivers	Good/Bad Day* Qual Symp* PedsQL+ PedsQL-C+ PRO-CTCAE (C) CQOLC FACIT-Sp-12 HADS HHI mP-PAM*	Good/Bad Day* Qual Symp* PedsQL+ PedsQL-C+ PRO-CTCAE (C)	Good/Bad Day* Qual Symp* PedsQL+ PedsQL-C+ PRO-CTCAE (C) CQOLC FACIT-Sp-12 HADS HHI mP-PAM*	Good/Bad Day* Qual Symp* PedsQL+ PedsQL-C+ PRO-CTCAE (C) CQOLC FACIT-Sp-12 HADS HHI mP-PAM*	Good/Bad Day* Qual Symp* PedsQL+ PedsQL-C+ PRO-CTCAE (C) CQOLC FACIT-Sp-12 HADS HHI mP-PAM*

Good/Bad Day = Good Day Bad Day Scale, Qual Symp = Qualitative Symptoms Assessment, PedsQL = Pediatric Quality of Life Inventory V. 4, PedsQL-C = PedsQL Cancer Module V. 3, PRO-CTCAE (P) = PRO-CTCAE Pediatric Form, PRO-CTCAE (A) = PRO-CTCAE Adult Form, PRO-CTCAE (C) = PRO-CTCAE Caregiver Proxy Form, CQOLC = Caregiver Quality of Life Index – Cancer, FACIT-Sp-12 = Functional Assessment of Chronic Illness Therapy – Spiritual Well-Being Subscale, HADS = Hospital Anxiety and Depression Index, HHI = Herth Hope Index, mP-PAM = Modified Patient-Parent Activation Measure

**Not included for Spanish speaking patients and families.*

+Used only for caregivers of children aged 2 years and older.