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STUDY TITLE: Phase I Clinical Trial of Anti-CD19/20/22 Chimeric Antigen Receptor T Cells for Treatment of Relapsed or Refractory Lymphoid Malignancies (Non-Hodgkin Lymphoma, Acute Lymphoblastic Leukemia, Chronic Lymphocytic Leukemia, B-Prolymphocytic Leukemia)

PRINCIPAL INVESTIGATOR:

Sumithira Vasu, MBBS
Ohio State University Comprehensive Cancer Center
Arthur G. James Cancer Hospital
1130C Lincoln Tower,
Columbus, OH 43210
Sumithira.Vasu@osumc.edu
(614) 293-8197

IND SPONSOR:

Marcos de Lima, MD
Ohio State University Comprehensive Cancer Center
Arthur G. James Cancer Hospital
1130C Lincoln Tower,
Columbus, OH 43210
Marcos.deLima@osumc.edu

SUBSITES:

Margaret Lamb, MD
Pediatric Hematology/Oncology/BMT
Nationwide Children's Hospital
700 Children's Drive
Columbus, OH 43205
Margaret.Lamb2@nationwidechildrens.org

STUDY SCHEMA

A. Non-Hodgkin Lymphoma with lesions ≤ 5 cm, indolent lymphomas, Chronic Lymphocytic Leukemia (without Richter's transformation), or B-Prolymphocytic Leukemia with lesions ≤ 5 cm (not including splenomegaly) Cohort (Cohort A)

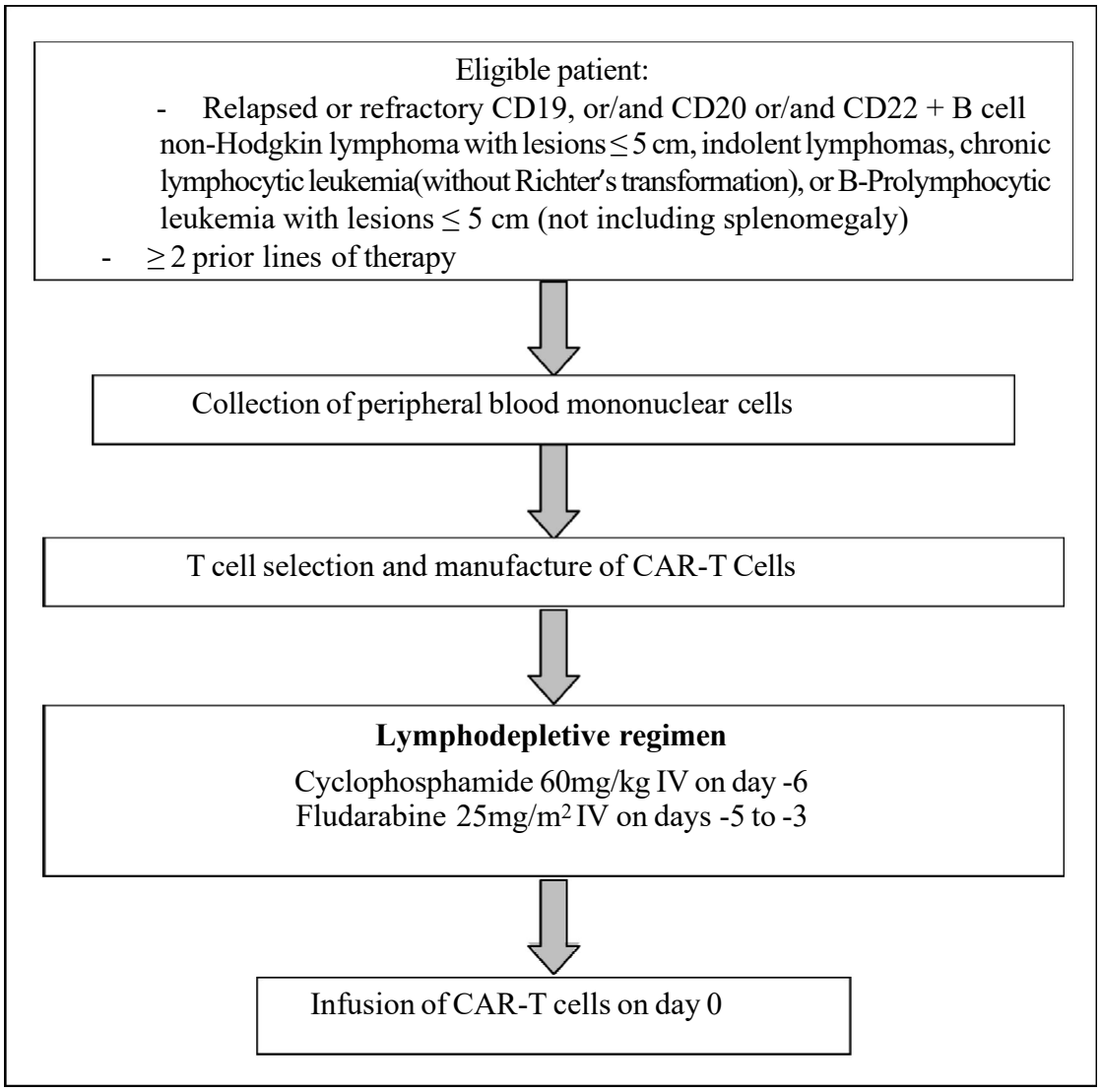


Figure 1: Study Schema for Cohort A.

Notes: Dates based on 8-day manufacturing schedule. A 6 to 12-day manufacturing schedule may be necessary to achieve planned dose. Dates will be adjusted.

B. Acute Lymphoblastic Leukemia, Lymphoid blast crisis from Chronic myeloid leukemia, Chronic Lymphocytic Leukemia (with Richter's transformation), Non-Hodgkin lymphoma with lesions > 5 cm and/or lymphoblastic lymphoma, Non-Hodgkin lymphoma with circulating lymphoma cells, or B-Pro lymphocytic leukemia with lesions > 5 cm (not including splenomegaly) Cohort (Cohort B)

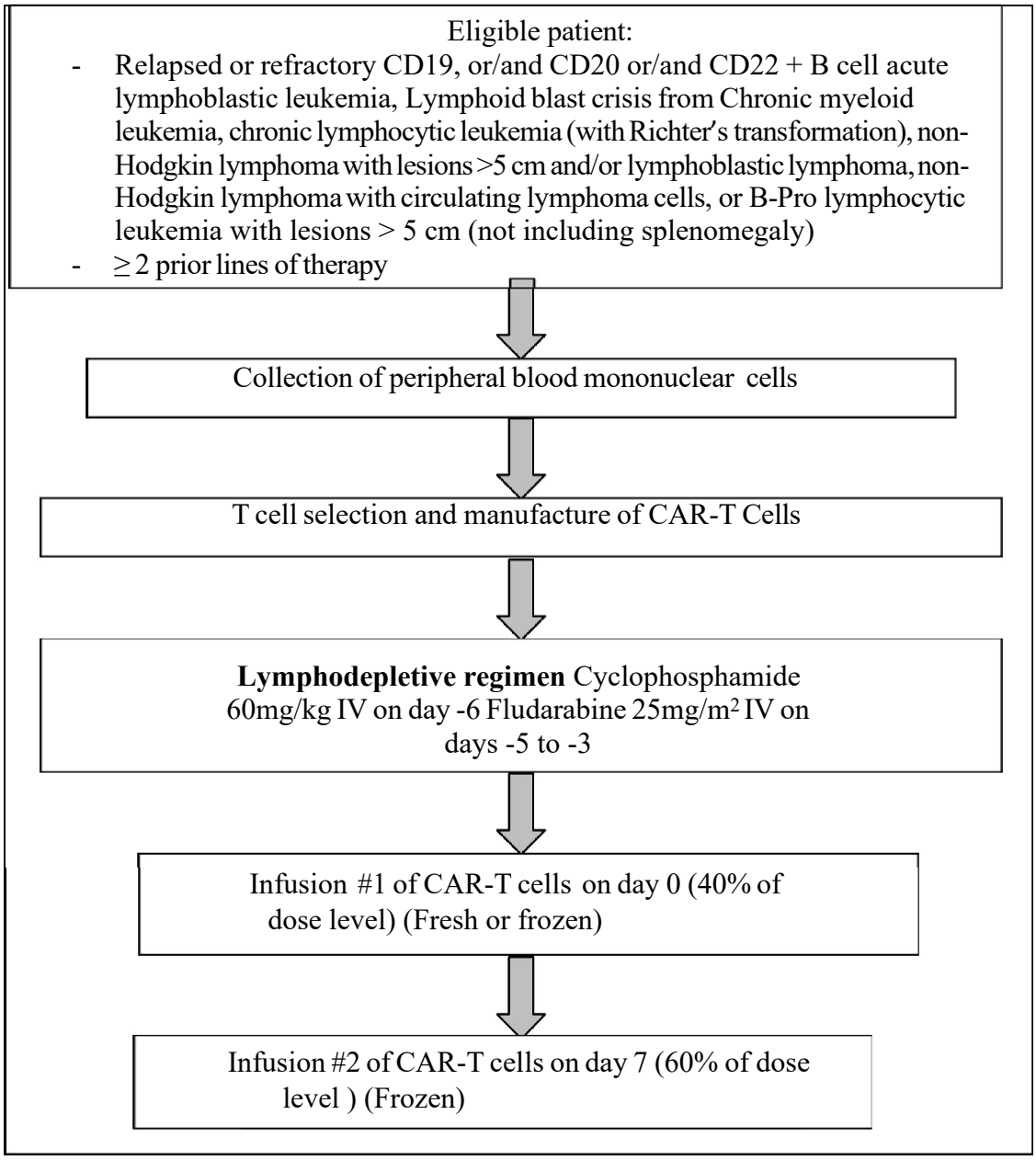


Figure 2: Study Schema Cohort B.

Notes: Dates based on 8-day manufacturing schedule. A 6 to 12-day manufacturing schedule may be necessary to achieve planned dose. Dates will be adjusted.

C. Pediatric and Young Adults (Age ≥ 2-25.99) Acute Lymphoblastic Leukemia, Non-Hodgkin Lymphoma Cohort (Cohort C)

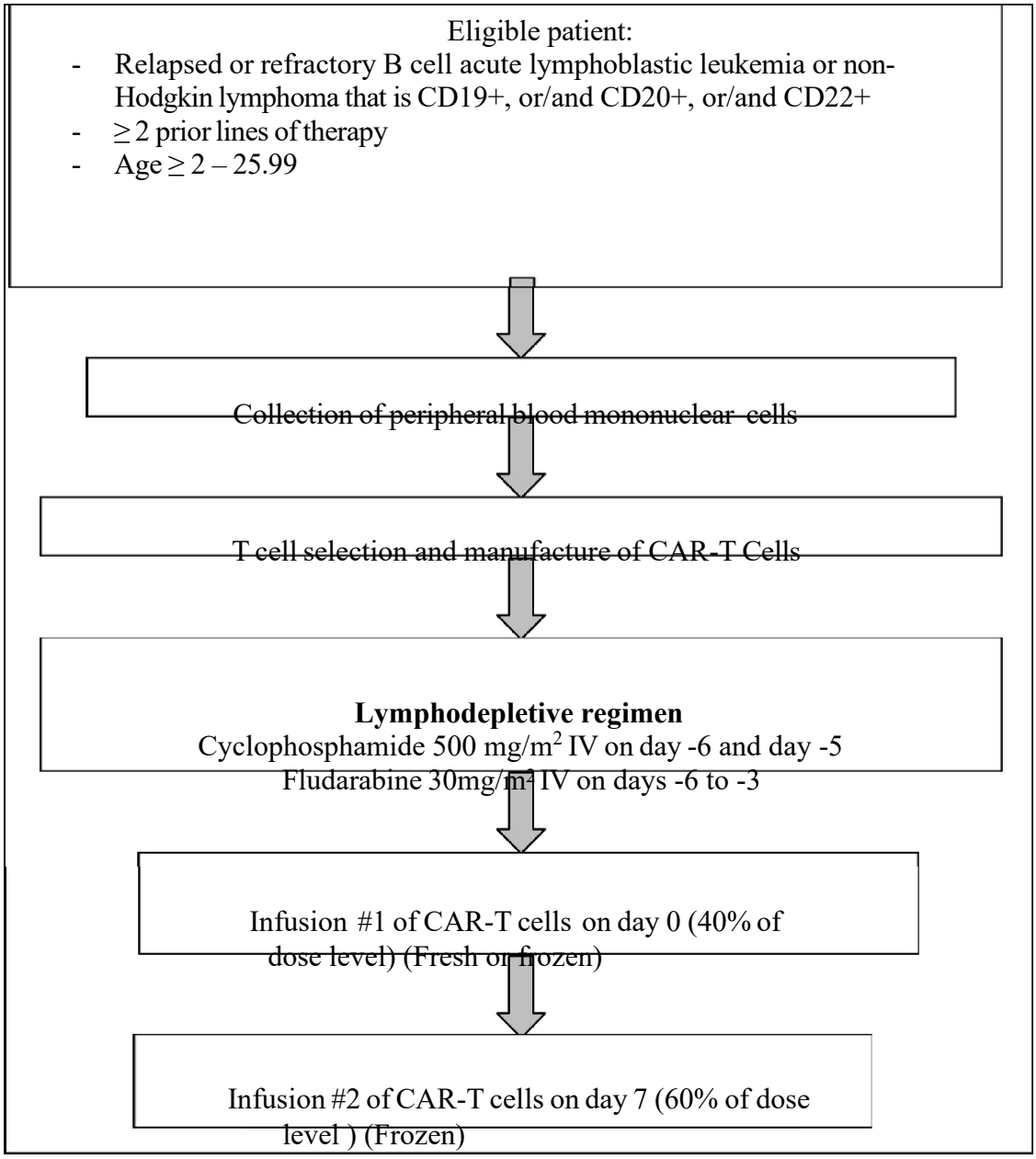
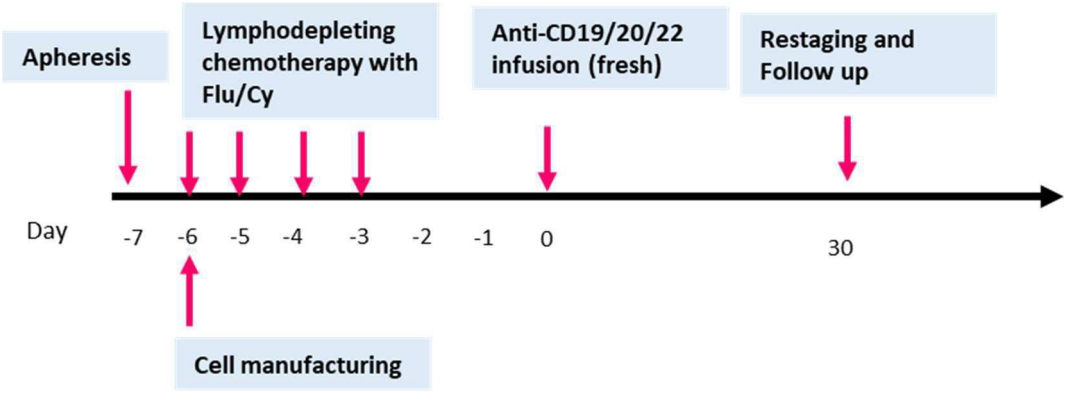


Figure 3: Study Schema Cohort C.

Notes: Treatment days are based on 8-day manufacturing schedule. A 6 to 12-day manufacturing schedule is allowed as needed to achieve planned dose. Days between completion of chemotherapy and the infusion will be adjusted as needed.

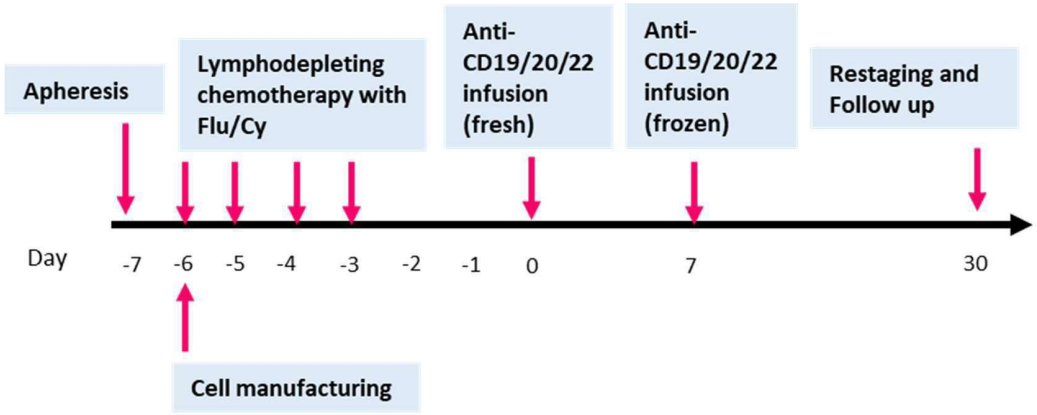
CAR-T CELL MANUFACTURE AND TREATMENT SCHEMA

A. Cohort A



* Product freezing will be done if patient conditions require a delay in conditioning and/or infusion of CAR-T cell product

B. Cohort B and Cohort C



* Product freezing will be done if patient conditions require a delay in conditioning and/or infusion of CAR-T cell product

PROTOCOL SUMMARY

Protocol Number/Title	Phase I Clinical Trial of Anti-CD19/20/22 Chimeric Antigen Receptor T Cells for Treatment of Relapsed or Refractory Lymphoid Malignancies (Non-Hodgkin Lymphoma, Acute Lymphoblastic Leukemia, Lymphoid blast crisis from Chronic myeloid leukemia Chronic Lymphocytic Leukemia, B-Prolymphocytic Leukemia)
Study Phase	Phase I
Brief Background/Rationale	This study seeks to determine the safety of the infusion of autologous T cells that have been modified through the introduction of a human chimeric antigen receptor targeting the B cell surface antigen CD19/20/22
Primary Objective	To determine the safety of the treatment of relapsed or refractory Non-Hodgkin lymphoma, relapsed/refractory chronic lymphocytic leukemia, refractory B-Prolymphocytic leukemia, Lymphoid blast crisis from Chronic myeloid leukemia and relapsed/refractory acute lymphoblastic leukemia with chimeric antigen receptor T cells targeting CD19/20/22 and to find the recommended phase II dose for this cellular therapy
Secondary Objective(s)	• To describe the safety profile of the infusion of CAR-T cells targeting CD19/20/22 in relapsed/refractory Non-Hodgkin lymphoma, relapsed/refractory chronic lymphocytic leukemia, refractory B-Prolymphocytic leukemia, and relapsed/refractory acute lymphoblastic leukemia. • To describe the toxicities related to infusion of CAR-T cells targeting CD19/20/22. • To describe the overall response rate and complete response rate of relapsed B cell malignancies treated with CAR-T cells targeting CD19/20/22.
Correlative Objective(s)	• To describe the persistence of Anti-CD19/20/22 CAR-T cells, measured by flow cytometry and qPCR; • To describe the T cell subpopulations of the Anti-CD19/20/22 CAR-T cell product before infusion; • To describe the changes in Anti-CD19/20/22 CAR-T cells after infusion and their correlation with disease response and adverse events; • To investigate the correlation between changes in cytokine plasma concentrations • To investigate the changes in Anti-CD19/20/22 CAR-T cell changes over time, including cell subpopulations, gene expression and proteomics.

Sample Size	Up to 54 subjects total (including dose escalation). Up to 60 subjects may be screened to account for screen failures.
Disease sites/Conditions	Non-Hodgkin Lymphoma Acute Lymphoblastic Leukemia Chronic Lymphocytic Leukemia B-Prolymphocytic leukemia Lymphoid blast crisis from Chronic myeloid leukemia
Interventions	Autologous chimeric antigen receptor T cells targeting CD19/20/22, single infusion (Cohort A), split infusion (Cohort B and Cohort C)
	Cyclophosphamide 60mg/kg on day -6 (500 mg/m^2 on days -6 and -5 for age < 18y. Pediatric lymphodepletive chemotherapy regimen is described in Appendix 7.)
	Fludarabine 25mg/m^2 daily on days -5 to -3 (30 mg/m^2 on days -6 through -3 for age < 18y. Pediatric lymphodepletive chemotherapy regimen is described in Appendix 7.)

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1 INTRODUCTION

1.1 Chimeric antigen receptor-modified (CAR)-T cells

Chimeric antigen receptors (CAR) are recombinant T cell receptors composed of an extracellular fragment derived from immunoglobulin variable fragment, as single chain (scFv), which is in turn linked to intracellular signaling sequences that are derived from T cells.[1] Insertion of a CAR in a T cell can induce activation of the T cell upon ligation of the scFv with its target antigen. CAR-T cells have instituted a paradigm shift in cancer immunotherapy.[2]

1.1.1 CD19 Specific CAR-T

Successful results of several pivotal studies led to FDA approval and CD19 targeted CAR-T is now commercially available for Non-Hodgkin's lymphoma and B-Acute lymphoblastic leukemia.[3]

1.1.2 Non-Hodgkin's Lymphoma

Non-Hodgkin lymphoma (NHL) represents the seventh most common malignancy in adults, with an estimated 72,240 new cases expected to be diagnosed in the United States in 2017.[4] Neelapu and colleagues[5] recently reported the multicenter experience with anti-CD19 Axicabtagene ciloleucel (axi-cel®, Kite Pharma) (n=111). Response rate was 82%, with a 54% CR rate. Summary of pivotal studies and response rates are summarized in Table 1.1-1 below.[3,5-7]

Table 1.1-1 Summary of Pivotal Studies in NHL

	Tisagenlecleucel (95)	Axicabtagene ciloleucel (56)	Lisocabtagene maraleucel (69)
Construct	Anti-CD19–4-1BB–CD3ζ	Anti-CD19–CD28–CD3ζ	Anti-CD19–4-1BB–CD3ζ
Follow-up, months	24	27.1	12
Median prior therapies	3	3	3
Overall response	54% ORR, 40% CR	83% ORR, 58% CR	73% ORR, 53% CR
Median OS, months	10.3, not reached for patients in CR	Not reached	Not reached
PFS at 2 years, %			
All patients	Not reported	39	Not reported
In CR	78	72	Not reported
Grade 3–4 AEs	23% CRS, 11% NT	12% CRS, 31% NT	2% CRS, 10% NT
CRS grading scale used	Penn	Lee	Lee
Treatment locale	Inpatient or outpatient	Inpatient only	Inpatient or outpatient
Approval status	FDA-approved for pediatric ALL and adult R/R DLBCL	FDA-approved for adult R/R DLBCL and PMBL	Not yet approved

AEs, adverse events; CR, complete response; NT, neurotoxicity; ORR, overall response rate; OS, overall survival; PFS, progression-free survival; PMBL, primary mediastinal B cell lymphoma; R/R, relapsed/refractory.

1.1.3 B-Acute lymphoblastic leukemia (B-ALL) and Lymphoid blast crisis from Chronic myeloid leukemia (CML)

Incidence of B-ALL is approximately 6000 new cases per year in the US. This disease occurs predominantly in children and young adults with younger patients having significantly better outcomes with survival rates of 90%. Older individuals diagnosed with ALL tend to fare poorly and approximately one third of patients will have long-term disease control. Table 1.1-2 provides a summary of CAR-T cell therapies in B-ALL. High rates of CR have been observed with 12 month event-free survival ranging from 50-66%.[8-14] The current standard of care is a consolidative allograft once patient achieves remission with CAR-T.

Patients with Lymphoid Blast Crisis of Chronic Myeloid Leukemia behave in similar ways to those with ALL and are treated with multiagent chemotherapy just like B-ALL. However, they have not been eligible to receive CAR T cells. Their phenotype is similar as well, and often express CD19, CD20 and or CD22.

Table 1.1-2 Summary of CAR-T Therapies in B-ALL

References	Age	Product	Co-stim	No	CRS*	sCRS*	ICANS*	sICANS*	CR	Prolonged response
Lee et al. (26)	Ped+YA	CD28 containing CAR	CD-28	51	N/A	7 (14%)	N/A	5 (10%)	61%	Median LFS[$], 18 mo
Gardner et al. (27)	Ped+YA	1:1 CD4:CD8	CD28+ 41BB	43	40 (90%)	10 (23%)	21 (49%)	9 (21%)	93%	12-mo EFS, 50.8%
Maude et al. (3)	Ped+YA	Tisagenlecleucel	41BB	75	58 (77%)	35 (44%)	30 (40%)	10 (13%)	81%	50%
Pasquini et al. (28)[#]	Ped+YA	Tisagenlecleucel	41BB	144	85 (59%)	19 (13%)	42 (29%)	12 (8%)	89%	6-mo LFS, 66%
Turtle et al. (25)	Adults	1:1 CD4:CD8	CD-28 + 41BB	30	25 (83%)	7 (23%)	15 (50%)	15 (50%)	90%	43% at 6 mo
Hay et al. (29)	Adults	1:1 CD4:CD8	CD28 + 41BB	47[@]	35 (70%)	4 (12%)	11 (40%)	14 (21%)	NA	NA
Park et al. (23)	Adults	MSK CAR-T	CD28	53	45 (85%)	14 (26%)	24 (46%)	22 (42%)	83%	Median EFS, 6.1 mo
Shah et al. (30)	Adults	KTE-X19	CD28	45	N/A	13 (29%)	N/A	17 (38%)	73%	Median EFS, 15 mo

*Different trials used different toxicity grading systems.
[$]Among responders.
[#]Post-marketing CIBMTR data.
[@]47 ALL patients of 133 patients with CD19 malignancies.
ALL, acute lymphoblastic leukemia; CRS, cytokine release syndrome; ICANS, immune effector cell-associated neurotoxicity syndrome; Ped+YA, pediatrics and young adults; Co-stim, costimulatory domain; CR, complete response; CIBMTR, Center for International Blood and Marrow Transplant Research.

1.1.4 Chronic lymphocytic leukemia and B-prolymphocytic leukemia

The first reports of successful clinical activity of anti-CD19 CAR-T cells were those of CLL responses; Porter and colleagues reported the achievement of remissions in CLL in 2011.[15] Updated results showed that heavily pretreated CLL patients achieved a response rate of 57% with anti-CD19 CAR-T cells with CD3ζ and 4-1BB costimulatory domains[15], with 8 out of 14 patients responding, 4 of whom had complete remission. In a separate trial with anti-CD19 CAR-T cells done at the National Cancer Institute (NCI), 7/8 patients presented a complete response.[16] Turtle and coworkers conducted a study with this fixed ratio product in CLL patients who had progressed after ibrutinib therapy, with an overall response rate of 76%.[13,17] Refractory B-Prolymphocytic leukemia (B-PLL) is a condition that may benefit from CAR T therapy and will be eligible for treatment in this study.

1.1.5 CD22 targeting in B cell malignancies

CD19-targeting CAR T-cell therapies have improved outcomes for ALL patients, however, the majority of these patients relapse, often with mutations in CD19 or loss of expression of CD19.[8] CD22 is also expressed in most cases of B-ALL and is usually retained following

CD19 loss.[18-20] Thus, CAR was expanded to target an alternative antigen CD22 CAR T cells as a salvage therapy for relapsed/refractory to CD19 targeted therapies.[21,22] CD22 CAR T was tested in a phase 1 dose escalation trial in children and young adults.[23] The trial included fifty-eight patients (median age, 17.5 years). Prior therapy included CD19-targeted therapy in 51 (87.9%) patients. To enhance CAR T-cell manufacturing feasibility and reduce interpatient product variability, CD4/CD8 T-cell selection was done of the apheresis material. The initial dose escalation was with DL2 (1 x 10^6/kg) expanded to (n =18) patients. The patients experienced increased inflammatory responses and hence the dose was de-escalated for remaining patients to DL1-TCS (3 x 10^5/kg; n = 25). Complete remission was achieved in 70% of the patients. Fifty (86.2%) of patients experienced cytokine release syndrome (CRS) which was mostly grade 1-2. Neurotoxicity was minimal and transient. Hemophagocytic lymphohistiocytosis–like toxicities were seen in 19/58 (32.8%) of patients, prompting utilization of anakinra.[24] The median overall survival was 6.0 months and thirteen patients proceeded to stem-cell transplantation. In summary, this trial confirms CD22 CAR T cells as a highly effective salvage option and provided foundation for combinations of targeted immunotherapy using CAR constructs for B-cell malignancies.

1.1.6 Bispecific targeting of CAR-T cells

CD19-, CD20- targeted CAR-T cells were evaluated in B-cell malignancies. The complete remission rate to all dose levels was 82% and for the highest dose, level of 2.5 x 10e6/kg was 92%. Four out of the 12 patients who achieved complete remission at the highest dose level relapsed ≥ 6 months post CAR-T infusion. In patients who relapsed, CD19 antigen expression was preserved.[25] Spiegel et al evaluated Bispecific CAR-T cells targeting CD19 and CD22 in patients who failed commercial CAR-T.[26] Out of 17 B-ALL and 22 NHL patients, relapses were seen in 10 and 14 patients respectively. 50% of relapses in the B-ALL cohort and 29% of patients with NHL were CD negative or low at relapse following Bispecific targeting of CD19 and CD22. These trials show that Bispecific targeting is safe and can be effective in B-cell malignancies.

1.2 Preliminary Data

1.2.1 Preclinical data for efficacy of CD19/20/22 Trispecific CAR T

Schneider et al [27] evaluated CAR T targeting CD19, 20 and 22 (Miltenyi, Lentigen). DuoCARS targeting CD19 and CD20 were linked to a second CAR targeting CD22. In vivo studies with B cell lymphoma lines showed Trispecific CAR T induced potent tumor clearance in tumors positive for all three antigens or had only one antigen, while the mono specific CAR Ts cells failed to prevent progression. The distinct signaling characteristics of the intracellular domains may contribute to complementary anti-tumor effects. The figures shown here reveal the anti-tumor efficacy of monospecific CAR Ts (M19 indicates anti CD19, M20 is anti CD20 and M22 is anti CD22). D1, D2, D3 and D4 are the Trispecific CARS targeting CD19, 20, 22. Briefly Tandem CARs with the CD19-CD20 targeting domain with one of the following costimulatory domains: OX40, ICOS, CD27, CD28 or 4-1BB were first developed.[27] After extensive in vitro and in vivo evaluation of OX 40 domain was found to be optimal in terms of cytotoxicity. Lentiviral vectors encoding each of these two CAR

constructs were screened for antitumor activity as cotransduction pairs, where each pair had one tandem CD19-20 CAR and one mono CD22 CAR. Figure 1.2 shows the bioluminescence assays of mice bearing Raji cells with all three antigens. Monospecific CARs do not prevent tumor progression whereas Trispecific CARs result in robust tumor clearance. These data show that Trispecific CARs do not result in antigen escape as a mechanism for tumor progression.

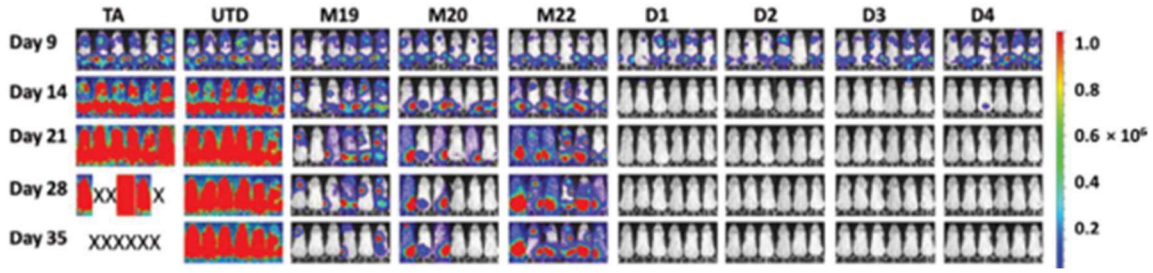

Figure 1.2: Trispecific CAR T cells are D1, D2, D3 and D4.

1.2.2 Point-of-care manufacturing of CAR-T

Commercial CAR-T relies on a central manufacturing paradigm and reliance on core academic center to be able to manage the complex logistics of manufacturing and supportive care of toxicities.[28] Automated manufacturing has shown to generate a consistent CAR-T product in terms of phenotype and viability.[29] Maschan et al reported on multi-center point-of-care manufacturing of CD19 CAR-T.[30] 54 patients were treated at 2 different sites with CR rates of 89% for B-ALL and 73% for NHL. With a median follow-up of 17 months, one-year survival rate in patients with B-ALL is 79% with a 10-month median duration of response; in NHL patients, one year survival is 92% and median duration of response has not been reached. This study showed that consistent CAR-T products in phenotype produced at geographic disparate locations resulted in a safe and effective treatment for B- cell malignancies. Other sites have also consistently used the Miltenyi Prodigy and TransAct manufacturing technology with a consistent CAR-T product yield, showing feasibility of this approach.[25,26]

1.3 Unmet needs in the era of commercially available CAR-T therapy

Lack of durable remissions and disease relapse remains a serious challenge despite the promise of commercial CAR-T therapy. Real-world data of commercially available products shows that 116 patients treated with Commercial CAR-T.[31] With a median follow-up of 8.2 months, 55 patients failed treatment; 27 (49%) were early progressors. The estimated 12-month progression-free survival (PFS) and overall survival (OS) were 47.2% (95% confidence interval [CI], 38.0-58.6) and 67.0% (95% CI, 57-79), respectively.[31]

Patterns of relapse have been described in detail in B ALL setting[8]: 1. **Antigen-negative relapse**: This occurs due to selective immune pressure from the CAR-T resulting in down regulation of target antigen.[32] Whether target antigen density determines response to CAR-T has been evaluated.[33-36] 2. **Antigen-positive relapse**: These are associated with reduced

persistence of CAR-T or an exhaustion phenotype of CAR-T. There is an unmet need to develop engineering strategies[37-39] that lead to broader target recognition and longer persistence of CAR-T. OX40 signaling has been shown to enhance persistence and function of CAR-T cells.[40-43]

Minimizing toxicity of CAR-T Therapy: Over the last decade, several studies have elucidated the biology and mechanisms of cytokine release syndrome[44-47] and neurotoxicity.[24,48-50] Several studies have evaluated use of prophylactic tocilizumab as minimizing toxicity while preserving antileukemic efficacy.[51,52] Other agents such as anakinra are also being evaluated in pre-emotive treatment of CAR-T associated toxicities.

Product characterization and mechanisms of safety and efficacy: Several studies have evaluated whether a defined CD4: CD8 contribution in product can influence safety and toxicity.[24,53] Products with stem or memory phenotype drive persistence and proliferation of T cells. [54,55]

1.4 Rationale for proposed trial

Relapse occurs in over 50% of patients treated with commercial CD19 directed CAR-T.[12,31] Relapses occur due to antigen escape from selective immune pressure either on the target antigen or due to exhaustion and lack of persistence of CAR-T cells.[56] Bispecific CAR-T cells targeting CD19 and CD20 or CD19 and CD22 have been shown to be efficacious and safe in patients with B-cell malignancies. Here we propose a first-in-human trial evaluating a novel CAR-T targeting three antigens: CD19, CD20 and CD22 along with a novel OX-40 costimulatory domain[42,43,57] that is hypothesized to lead to long-term persistence of CAR-Ts. We hypothesize that this CAR-T construct will be safe and effective in patients with B-cell malignancies. Carefully designed correlative studies and secondary endpoints will clarify whether Trispecific antigen targeting decreases incidence of antigen negative escape and whether the novel costimulatory domain influences persistence of CAR-T cells.

1.5 Rationale for pediatric cohort

Despite encouraging response rates in pediatric patients with B cell ALL receiving CD19 CAR T cells, 10-20% of patients will fail to achieve remission and 30–50% of those who achieve remission will relapse. The primary mechanism of relapse after CD19 CAR T cell therapy is CD19 antigen escape and 25% of pediatric ALL patients will relapse with CD19 negative blasts.[11] Strategies to prevent antigen escape are essential to improve durable remissions in this population. Moreover, there is an unmet need in pediatric relapsed/refractory B-cell lymphoblastic lymphoma as there is no FDA-approved CAR T cell product for this population. The goal of this study is to improve response rates and decrease antigen escape by incorporating multi-antigen targeting with tri-specific CD19/20/22 CAR T cells in B cell leukemia and lymphoma.

The FDA-approved CD19 CAR T cell product in pediatric ALL has a complete response rate of >80% and is the standard of care for relapsed/refractory CD19 positive leukemia; therefore, we propose to include pediatric patients with prior anti-CD19 CAR T cell

immunotherapy. Patients who have undergone prior anti-CD19 CAR therapy will be eligible if it has been at least 30 days since previous CAR T cell therapy and < 5% of circulating levels of CD3+ cells express the anti-CD19 CAR by flow cytometry. This is consistent with other ongoing and published CAR T cells clinical trials (NCT03241940, NCT05442515, NCT03448393, NCT04088890).

2 OBJECTIVES

2.1 Primary Objective

- To determine the safety of the treatment of relapsed/refractory Non-Hodgkin lymphoma, relapsed/refractory chronic lymphocytic leukemia, refractory B-Prolymphocytic leukemia, lymphoid blast crisis from chronic myeloid leukemia and relapsed/refractory acute lymphoblastic leukemia with chimeric antigen receptor T cells targeting CD19/20/22 and to find the recommended phase II dose for this cellular therapy.

2.2 Secondary Objectives

- To describe the safety profile of the infusion of CAR-T cells targeting CD19/20/22 in relapsed/refractory Non-Hodgkin lymphoma, relapsed/refractory chronic lymphocytic leukemia, refractory B-Prolymphocytic leukemia, lymphoid blast crisis from chronic myeloid leukemia and in relapsed/refractory acute lymphoblastic leukemia.
- To describe the toxicities related to infusion of CAR-T cells targeting CD19/20/22.
- To describe the overall response rate and complete response rate of relapsed B cell malignancies treated with CAR-T cells targeting CD19/20/22.
- To describe the overall and progression free survival of patients with relapsed lymphoma, CLL, B-PLL and ALL treated with Anti-CD19/20/22 CAR-T cells.

2.3 Correlative Objectives

- To describe the persistence of Anti-CD19/20/22 CAR-T cells, measured by flow cytometry and qPCR.
- To describe the T cell subpopulations of the Anti-CD19/20/22 CAR-T cell product before infusion.
- To describe the changes in Anti-CD19/20/22 CAR-T cells after infusion and their correlation with disease response and adverse events.
- To investigate the correlation between changes in cytokine plasma concentrations and changes in Anti-CD19/20/22 CAR-T cell subpopulations over time.
- To investigate proteomic changes in Anti-CD19/20/22 CAR-T cell subpopulations over time.
- To investigate whether antigen escape occurs in patients treated with Anti-CD19/20/22 CAR-T.

2.4 Study Endpoints

- Primary: Recommended phase II dose of Anti-CD19/20/22 CAR-T cells for each study group (Cohort A, Cohort B, and Cohort C).

- Secondary: 1) Toxicity profile for infusion of CAR-T cells targeting CD19; 2) Overall response rate of relapsed/refractory B cell malignancies treated with CAR-T cells targeting CD19/20/22; 3) Complete response rate of relapsed/refractory B cell malignancies treated with CAR-T cells targeting CD19/20/22; 4) Overall and progression free survival from time of infusion of Anti-CD19/20/22 CAR-T cells.

- Correlative: 1) Correlation between cytokine serum concentrations before and after treatment with Anti-CD19/20/22 CAR-T cells and disease response (overall and complete response rate); 2) Presence of measurable CAR-T cells after infusion, at 30, 60, 90 days and 6 and 12 months; 3) Correlation between CD19/20/22 expression on tissue and disease response.

3 STUDY DESIGN

3.1 Study design including dose escalation / cohorts

This study is a Parallel Group Phase I trial. Adult subjects with CD19, and/or CD20, and/or CD22+ lymphomas and chronic lymphocytic leukemia will enroll in Cohort A (NHL with lesions ≤ 5 cm, Indolent lymphomas, B-PLL with lesions ≤ 5 cm (not including splenomegaly) and CLL without Richter's transformation), while adult patients with Lymphoid blast crisis from CML, ALL, CLL with Richter's transformation, NHL with lesions > 5 cm, lymphoblastic lymphoma, NHL with circulating lymphoma cells, or B-PLL with lesions > 5 cm (not including splenomegaly) will enroll in Cohort B. Pediatric subjects with CD19, and/or CD20, and/or CD22+ ALL or NHL will enroll in Cohort C. Dose escalation in each group will be conducted in parallel under a "3+ 3" phase I study design. In each group, patients will be enrolled sequentially to each dose level defined in Tables 3.1 and 3.2, starting with 5 x 10^5 CAR T cells/kg for Cohort A and Cohort B. As 5 x 10^5 CAR T cells/kg was initiated in Cohort and A and B and deemed safe in adults prior to adding Cohort C, the starting dose will be 1 x 10^6 cells/kg for Cohort C. The CAR-T cell dose escalation schemas are presented in Table 3.1 and Table 3.2.

Table 3.1. Cohort A and B Dosing Schema

Dose Level	Anti-CD19/20/22 CAR-T Cell Dose *€	Fludarabine (mg/m^2 from day -5 to -3)	Cyclophosphamide (mg/kg/IV on day -6)
Level -1	1 x 10^5 cells/kg	25	60
Level 1 [Starting Dose]	5 x 10^5 cells/kg	25	60
Level 2	1 x 10^6 cells/kg	25	60
Level 3	2 x 10^6 cells/kg	25	60

*CAR-T cell dose will be at or as close as possible to the pre-specified dose level, with a ± 10% variation.

€ For Cohort B patients, this value corresponds to the total CAR-T cell dose infused, divided in two doses, dose 1 (day 0) is 40% of the total dose and dose 2 (day 7) is 60% of total dose

* Mesna, will be administered in accordance with institutional standard operating procedures.

Table 3.2. Cohort C Dosing Schema

Dose Level	Anti-CD19/20/22 CAR-T Cell Dose *€	Fludarabine (mg/m^2 from day -6 to -3)	Cyclophosphamide (mg/m^2 on day -6 and day -5)
Level -1	5 x 10^5 cells/kg	30	500
Level 1 [Starting Dose]	1 x 10^6 cells/kg	30	500
Level 2	2 x 10^6 cells/kg	30	500

*CAR-T cell dose will be at or as close as possible to the pre-specified dose level, with a ± 10% variation.

€ For Cohort C patients, this value corresponds to the total CAR-T cell dose infused, divided in two doses, dose 1 (day 0) is 40% of the total dose and dose 2 (day 7) is 60% of total dose

* Mesna, will be administered in accordance with institutional standard operating procedures.

If none of the first 3 patients experiences a dose limiting toxicity (DLT), dose escalation may proceed, and subjects will be enrolled in the subsequent higher dose level. If 1/3 patient experiences a DLT, 3 additional subjects will be enrolled at the same dose level. If no further DLT is observed (1/6), dose escalation may proceed, and new subjects will enroll at the subsequent higher dose level. However, if 2 or more subjects in a cohort experience DLT, enrollment will be done at the immediately lower dose level (level -1 for the initial cohort). If no further DLT is observed or if a maximum of 1/6 subjects experience DLT, then dose re-escalation may be explored with new subjects entered again at a prior dose level. If dose de-escalation requires enrollment of subjects at dose level -1 and 2/6 or more DLTs occur at this dose level, no further patients will be enrolled to the study. In cases of re-escalation, a total of 6 additional subjects will be enrolled in a re-escalation dose level after subject safety events are evaluated by the Principal Investigator and the IND Sponsor. The maximum tolerated dose (MTD) is therefore defined as the dose level immediately below that in which ≥ 2/6 subjects experience a DLT. A total of 6 patients will be enrolled at the MTD. Dose-limiting toxicity (DLT) is defined in Section 6.8.

Staggered Enrollment
Subjects will be enrolled in this trial in a staggered fashion. The first three subjects in each dose level will receive the planned Anti-CD19/20/22 CAR-T cell product infusion no sooner than 30 days from each other. Escalation to a subsequent dose level will occur only after 30 days have elapsed from the infusion of the last patient enrolled to the previous dose level. Staggered enrollment will occur independently for the two groups.

If de-escalation followed by re-escalation occurs (as delineated above), staggered enrollment continues, and subjects in the same cohort will receive the planned human Anti-CD19/20/22 CAR-T cell product infusion no sooner than 30 days from each other as described above. The same stopping rules apply to dose level -1.

Manufacturing Safety Review
A safety review of manufacturing success rate will be generated after 5 products have been manufactured, and shared with the FDA. The study is to be on hold if we fail to manufacture 1 or more products in the first 5 patients.

3.2 Number of Subjects

Each group will enroll a minimum number of 4 subjects if (2/3) dose–limiting toxicities were to be observed in the initial dose levels (level 1) and (2/3) dose–limiting toxicities were to be observed in dose level -1. The study would be closed because of excessive toxicity.

For Cohorts A and B, if no dose-limiting toxicities are observed in the 3 planned dose levels; 9 patients would be enrolled (three in each dose level). If 1/6 dose-limiting toxicities were to be observed in each planned dose level; a maximum number of 18 patients would be enrolled in the dose escalation part of the study (six in each dose level). The same number of subjects (18) would occur if 6 patients were enrolled in dose level 1, -1 and then back in 1 after re-escalation.

In Cohort C, if no dose-limiting toxicities are observed in the 2 planned dose levels (dose levels 1 and 2), 6 patients would be enrolled (three in each dose level). If 1/6 dose-limiting toxicities were to be observed in each planned dose level, a maximum of 12 patients would be enrolled in the dose escalation part of the study (six in each dose level). A maximum of 18 patients would be enrolled if 6 patients were enrolled in dose level 1, -1 and then back in 1 after re-escalation.

Considering these are three separate treatment groups, the minimum number of subjects enrolled in the complete study will be 12 (4 per cohort) and the maximum number in dose escalation will be 54 (18 per cohort). To account for potential screen failures, up to 60 subjects may be screened.

3.3 Replacement of Subjects

The primary objective of this study is to assess the safety of treatment and obtain the optimal Phase II dose for this cellular therapy. Therefore, patients who receive lymphodepleting chemotherapy but subsequently do not receive the product will not be replaced. However, patients who are enrolled, but do not start lymphodepleting chemotherapy for a variety of reasons will be replaced. These patients will be replaced with subjects meeting the criteria for the group in which the prior subject was enrolled, and at the same dose level. All inclusion and exclusion criteria will continue to apply to replacement subjects.

3.4 Duration of therapy and follow up

3.4.1 Duration of Therapy

This study involves infusion of Anti-CD19/20/22 CAR-T cells administered after an immunosuppressive conditioning regimen of fludarabine and cyclophosphamide. As such, the therapy is given only once but consists of several parts:

1. **Autologous lymphocyte/mononuclear cell collection**. Lymphocytes will be collected through standard apheresis procedures as per Standard Operating Procedures of Arthur G. James Cancer Hospital or Nationwide Children's Hospital. WBC and % CD3 will be measured within 1 week of cell collection. CD3% will only be measured if absolute lymphocyte count is <100μLSuggested blood volumes to collect based on absolute lymphocyte count have been provided to the apheresis department. If cryopreserved infusion is planned due to clinical indication or scheduling constraints, autologous apheresis collection can occur anytime between day - 30 and day -7. If fresh product infusion is planned, then autologous apheresis collection can occur anytime between day -12 and day -7.
2. **CAR-T cell manufacturing**. This procedure will occur over approximately 6-12 days.
3. **Lymphodepletion (6 days = 4 days of therapy and 2 days of rest)**. 4 days of immunosuppressive chemotherapy. Cyclophosphamide given at a dose of 60mg/kg/IV on day -6 and Fludarabine 25mg/m^2/IV on days -5 to -3, inclusive. Fluids will be administered prior to initiation of cyclophosphamide. Treatment

plan to include antiemesis prophylaxis according to institutional standard operating procedures. Mesna, will be administered in accordance with institutional standard operating procedures. Pediatric lymphodepletive chemotherapy regimen is described in Appendix 7.

4. **CAR-T Cell Infusion.** The infusion of CAR-T cells targeting CD19/20/22 will occur over 5-30 minutes. In Cohort A patients, this is done on day 0. In Cohort B and Cohort C patients, this is done on days 0 and 7. NOTE: if conditions for day 7 infusion are not met, infusion can be delayed until day 10 providing all criteria are met.
5. **Early post-infusion.** From day 0 to day 30 after infusion. During this time, subjects will be observed closely for the development of acute symptoms and complications related to the infusion of CAR-T cells. At the end of this period (T+30 ±7 days) a response assessment will be performed.
6. **The long-term post – infusion period.** From day 30 through 12 months after infusion. During this period of time, patients will have clinical follow up as outlined in the study calendar and will have imaging studies to determine their disease status. Additional follow up will be done per their clinical manifestations.
7. **Long-term safety follow up.** From year 1 until year 15, when follow up for safety will continue.

Participation in this study will continue for the period of time specified unless one of the following occurs. Because the collection of T cells, the lymphodepletive regimen and infusion of CAR-T cells occurs only once, it is not possible to discontinue these interventions once they have occurred.

Off-treatment and off-study criteria are listed/detailed on Section 6.10 and 6.11, below.

The Sponsor reserves the right to temporarily suspend or prematurely discontinue this study. The date and reason for discontinuation must be documented. Every effort should be made to complete the appropriate assessments.

NOTE: In subjects who discontinue research-related testing (disease assessments, laboratory samples) after CAR-T cell infusion, investigators will attempt to continue with the long term safety follow up for monitoring of potential gene therapy – related delayed adverse events, as required per federal guidelines and as delineated in Section 3.4.2. In patients who discontinue their participation after CAR-T cell infusion for reasons other than death or decision to withdraw from the study, safety outcomes and correlative samples will continue to be collected as scheduled as long as subjects elect to continue participation in this part of the protocol (see study parameters, Section 11).

3.4.2 Duration of Follow Up

Patients will be followed for dose-limiting toxicity for 30 days after the day of infusion (day 0) of CAR-T cells or until death, whichever occurs first. Patients will be followed for survival, disease status, laboratory samples, and correlative markers until 12 months after CAR-T cell therapy.

The clinical course of each adverse event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

Follow up will be 15 years for monitoring for potential gene therapy – related delayed adverse events.

During the first 5 years after treatment with Anti-CD19/20/22 CAR-T cells, subject follow up will be done annually in clinical visits, in person, following the form delineated in Appendix 5, which will record the following information:

- Exposure to mutagenic agents;
- Exposure to medicinal products at the time of follow up contact;
- History;
- Physical examination;
- Laboratory test results including assay for persistent vector sequence (twice yearly);
- Emergence of new conditions:
    - New malignancy(ies)
    - New incidence or exacerbation of a pre-existing neurologic disorder
    - New incidence or exacerbation of a pre-existing rheumatologic or autoimmune disorder
    - New incidence of a hematologic disorder.

During the subsequent 10 years (years 6 – 15 after treatment with Anti-CD19/20/22 CAR-T cells), subjects will be contacted annually. If office visits are not scheduled, then patients may be contacted by telephone or written questionnaire. Contact throughout years 6 – 15 will be done following the form in Appendix 6 which will record the following information:

- Emergence of new conditions, including but not limited to:
    - New malignancy(ies)
    - New incidence or exacerbation of a pre-existing neurologic disorder
    - New incidence or exacerbation of a pre-existing rheumatologic or autoimmune disorder
    - New incidence of a hematologic disorder.

During years 6 – 15 after treatment, subjects with previous laboratory results indicating vector persistence will have this test repeated annually until resolution.

All subjects will be provided with the study coordinator contact information and encouraged to report delayed adverse events, including unexpected illness or hospitalization.

4 SUBJECT SELECTION

Each of the criteria in the sections that follow must be met in order for a subject to be considered eligible for this study. Use the eligibility criteria to confirm a subject's eligibility.

For patients who already have a cryopreserved product and had to come off study for any reason, they need to meet all eligibility criteria for re-enrollment, with the exception of Absolute lymphocyte count ≥ 100/µl. This criterion is not necessary since they already have a product.

4.1 Inclusion criteria

Eligible Diseases:

- Cohort A: Adult subjects with relapsed or refractory Non-Hodgkin Lymphoma with lesions ≤ 5 cm, indolent lymphomas, Chronic Lymphocytic Leukemia without Richter's transformation, or B-Prolymphocytic Leukemia with lesions ≤ 5 cm (not including splenomegaly)
- Cohort B: Adult subjects with lymphoid blast crisis, Acute Lymphoblastic Leukemia, Chronic Lymphocytic Leukemia with Richter's transformation, Non-Hodgkin lymphoma with lesions > 5 cm and/or lymphoblastic lymphoma, or Non-Hodgkin lymphoma with circulating lymphoma cells, B-Prolymphocytic leukemia with lesions > 5 cm (not including splenomegaly)
- Cohort C: Pediatric and young adult subjects (age ≥ 2 – 25.99) subjects with Acute Lymphoblastic Leukemia or Non-Hodgkin Lymphoma

Subjects must have been treated with at least two lines of therapy. Disease must have either progressed after the last regimen or presented failure to achieve complete remission with the last regimen. B-PLL is defined as having greater than 55% prolymphocytes in the peripheral blood.

Note: cohort assignment at discretion of PI depending on patient disease/ history

Detailed eligibility criteria:

- Subjects with relapsed/refractory CLL after at least 2 prior lines of appropriate therapy and must have previously received an approved BTK inhibitor and venetoclax.
- Subjects with refractory high-grade B-cell lymphoma who relapse within 12 months of autologous stem cell transplant.
- Subjects with relapsed/refractory B-Prolymphocytic leukemia who received at least 1-2 prior lines of appropriate therapy and who have failed or are ineligible for allogeneic stem cell transplant.
- Subjects with relapsed/refractory Acute B-lymphoblastic leukemia who received at least 2 prior lines of appropriate therapy or who have failed or are ineligible for allogeneic stem cell transplant.
- Subjects with relapsed/refractory lymphoid blast crisis from prior CML who received at least 2 prior lines of therapy (Tyrosine kinase inhibitors, multiagent chemotherapy) or have failed or are ineligible for allogeneic stem cell transplant

- The patient's lymphoid malignancy must be positive for at least one target antigen (CD19 and/**or** CD20 and/**or** CD22), either by immunohistochemistry or flow cytometry analysis on the last biopsy available or peripheral blood for circulating disease.

- Patients who received blinatumomab or inotuzumab are eligible.

- Patients who received prior CAR T-cells are eligible, (commercial CD 19 CAR-T cells or dual CAR-T cells), if it has been at least 30 days since previous CAR T cell therapy and <5% of circulating levels of CD3+ cells express the prior CAR by flow cytometry.

- Age ≥ 2 years.

- ECOG Performance status ≤ 2 (See Appendix 1). For patients < 16 years, Performance score Lansky ≥ 50 (See Appendix 2).

- Total bilirubin ≤ 1.5 times the institutional upper limit of normal for age

- AST (SGOT) ≤ 3 X institutional upper limit of normal for age

- ALT (SGPT) ≤ 3 X institutional upper limit of normal for age

- Creatinine clearance more than or equal to 50 ml/min calculated by the Cockcroft – Gault formula, or by Schwartz formula for patients < 18y

- Subjects must have adequate pulmonary function as defined as pulse oximetry ≥ 92% on room air.

- Subjects must have adequate cardiac function as defined as left ventricular ejection fraction ≥ 40% in the most recent echocardiogram.

- Absolute Lymphocyte Count ≥100/uL ; if WBC is low and differential is not performed, CD3 count should be ≥ 100/ul.

- Subjects (or legal guardians) must have the ability to understand and the willingness to sign a written informed consent document.

- For women of childbearing potential: agreement to remain abstinent (refrain from heterosexual intercourse) or use a contraceptive method with a failure rate of < 1% per year during the treatment period and for at least 6 months after the Anti-CD19/20/22 CAR-T cell infusion.
- A woman is considered to be of childbearing potential if she is postmenarcheal, has not reached a postmenopausal state (< 12 continuous months of amenorrhea with no identified cause other than menopause), and has not undergone surgical sterilization (removal of ovaries and/or uterus). Examples of contraceptive methods

with a failure rate of < 1% per year include bilateral tubal ligation, male sterilization, hormonal contraceptives that inhibit ovulation, hormone-releasing intrauterine devices, and copper intrauterine devices.

- The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

- For men: agreement to remain abstinent (refrain from heterosexual intercourse) or use contraceptive measures, and agreement to refrain from donating sperm, as defined below:
- With female partners of childbearing potential, men must remain abstinent or use a condom plus an additional contraceptive method that together result in a failure rate of < 1% per year during the treatment period and for at least 6 months after the Anti-CD19/20/22 CAR-T cell infusion. Men must refrain from donating sperm during this same period.
- With pregnant female partners, men must remain abstinent or use a condom during the treatment period and for at least 6 months after the human anti- CD19 CAR- T cell infusion to avoid potential embryonal or fetal exposure. The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the patient. Periodic abstinence (e.g., calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

4.2 Exclusion Criteria

The presence of any of the following will exclude a subject from study enrollment.

- Autologous transplant within 6 weeks of planned CAR-T cell infusion.

- Allogeneic stem cell transplant or donor lymphocyte infusion within 2 months of planned CAR-T cell infusion and patients must be off immunosuppressive agents. Patients with live vaccines given 28 days prior to LD chemotherapy will be excluded.

- Active graft versus host disease.

- Active central nervous system or meningeal involvement by lymphoma or leukemia.

  Subjects with untreated brain metastases/CNS disease will be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events. Patients with a history of CNS or meningeal involvement must be in a documented remission by CSF evaluation and contrast-enhanced MRI imaging for at least 90 days prior to registration.

- Active malignancy, other than non-melanoma skin cancer or carcinoma *in situ* (e.g. cervix, bladder, breast). Patients with a prior or concurrent malignancy whose

natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational regimen are eligible for this trial (e.g. Low Gleason score prostate Cancer).

- A minimum of 28 days must have elapsed between prior treatment with investigational agent(s) and the day of lymphocyte collection.

- HIV-seropositive patients are allowable, however must be on effective anti-retroviral therapy with undetectable viral load within 6 months of enrollment to be eligible for this trial.

- Subjects with uncontrolled intercurrent illness including, but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, pulmonary abnormalities or psychiatric illness/social situations that would limit compliance with study requirements.

- Pregnant or breastfeeding women are excluded from this study because CAR-T cell therapy may be associated with the potential for teratogenic or abortifacient effects. Women of childbearing potential must have a negative serum pregnancy test. Because there is an unknown, but potential risk for adverse events in nursing infants secondary to treatment of the mother with CAR-T cells, breastfeeding should be discontinued. These potential risks may also apply to other agents used in this study.

- Evidence of myelodysplasia or cytogenetic abnormality indicative of myelodysplasia on any bone marrow biopsy prior to initiation of therapy

- Patients with a positive Hepatitis B core antibody or surface antigen are at high risk for HBV reaction and will require entecavir/tenofivir prophylaxis or serial Hep B PCR monitoring at the direction of an infectious disease specialist. Duration of prophylaxis to correspond with detection of Anti-CD19/20/22 CAR T cells/viral vector copies in serum or continued evidence of B-cell aplasia such as reduced IVIG levels. No antiviral prophylaxis is indicated with Hepatitis C positivity with negative PCR.

- Patients with history of clinically relevant CNS pathology such as epilepsy, seizure disorders, paresis, aphasia, uncontrolled cerebrovascular disease, severe brain injuries, dementia and Parkinson's disease.

- History of autoimmune disease (i.e. rheumatoid arthritis, systemic lupus erythematosus) with requirement of immunosuppressive medication within 6 months.

- Live vaccines given in 28 days prior to lymphodepleting chemotherapy

4.2.1 Pre-lymphodepletion safety check

Subjects will undergo a pre-lymphodepletion safety check on day of starting chemotherapy. The objective of these criteria are to avoid infusion in patients with acutely heightened risk of

toxicity.

Patients must continue to meet eligibility criteria prior to initiation of lymphodepleting chemotherapy, with exception of the absolute lymphocyte count. This eligibility re- check can be done up to 48 hours prior to initiation of lymphodepletion.

The following findings at the pre-lymphodepletion safety check will require a delay in chemotherapy administration until resolved:

1. Symptoms, signs or laboratory markers of active infection or systemic inflammatory response.
2. Symptoms, signs or laboratory markers of an uncontrolled medical condition, including but not limited to decompensation of cardiac or pulmonary conditions. These changes exclude symptoms, signs or laboratory markers of disease progression, as long as eligibility criteria are met.

If the condition that leads to failure to meet eligibility criteria is considered irreversible by the principal investigator, patient participation in the study will be discontinued.

4.3 Pre-infusion safety check prior to day 0

Subjects will undergo a pre-infusion safety check on day 0 or -1. The objective of these criteria are to avoid infusion in patients with acutely heightened risk of toxicity.

Subjects must meet the following organ function criteria prior to CAR-T cell infusion:

- Total bilirubin ≤ 2 times the institutional upper limit of normal for age, unless bilirubin rise is due to Gilbert's syndrome (maximum 2 times normal) or of non – hepatic origin.
- AST (SGOT) and ALT (SGPT) ≤ 4 X institutional upper limit of normal for age
- Serum Creatinine ≤ 2 X the institutional upper limit of normal for age
- Subjects must have adequate pulmonary function as defined as pulse oximetry $\geq 92\%$ on room air.
- Absence of clinical signs, symptoms or laboratory markers of cardiac dysfunction.

The following findings at the pre – infusion safety check will require a delay in the infusion until resolved:

1. Use of corticosteroids within 3 days prior to day 0 infusion, with the exception of agents used for prevention of emesis during lymphodepleting chemotherapy.
2. Neurologic symptoms suggestive of an active central nervous system condition.

4.4 Pre-infusion safety check prior to day 7 (Cohort B and Cohort C only)

Cohort B and Cohort C subjects who experienced greater than grade 1 CRS and/or ICANS

will not be eligible for second infusion.

Cohort B and Cohort C patients will undergo a pre-infusion safety check on day 6 or 7. The objective of these criteria are to avoid infusion in patients with acutely heightened risk of toxicity.

Patients presenting any of the following findings will NOT receive a second Anti-CD19/20/22 CAR-T cell infusion.

1. Neurologic symptoms suggestive of an active central nervous system condition.
2. Signs or laboratory markers of active infection or systemic inflammatory response.
3. Patients with fever over 38.2 degrees Celsius.
4. CRS grade > 1 or ICANS > 1, regardless of resolution status.
5. CRS grade = 1 or ICANS = 1 that has not resolved at the time of day 7 -10 pre – infusion safety check. If cells are not given on day 7, pre infusion safety check will be done on day of infusion up to day 10.

Subjects must meet the following organ function criteria prior to CAR-T cell infusion:

- Total bilirubin ≤ 2 times the institutional upper limit of normal, unless bilirubin rise is due to Gilbert's syndrome (maximum 2 times normal) or of non – hepatic origin.
- AST (SGOT) and ALT (SGPT) ≤ 4 X institutional upper limit of normal
- Serum Creatinine ≤ 2 X the institutional upper limit of normal
- Subjects must have adequate pulmonary function as defined as pulse oximetry $\geq 92\%$ on room air.
- Absence of clinical signs, symptoms or laboratory markers of cardiac dysfunction.

4.5 Inclusion of Women and Minorities

Men, women and members of all races and ethnic groups are eligible for this trial.

5 REGISTRATION

For subsite patients, sites must send the signed consent form (and assent form if applicable), documentation of the consent process, and the Screening Form (refer to Supplemental Forms Document) within 2 business days of initial consent.

Patients will be registered after meeting all entry requirements and signing of the informed consent document.

OSU patients will be registered by the OSU research coordinator, as per CTO standard practice. Nationwide Children's Hospital (NCH) subsite patients will have eligibility verified and will be entered on study centrally at The Ohio State University by the Multi-Center Trial

Program (MCTP). The Multi-Center trial program is the operational and regulatory framework for trials conducted at both institutions. The subsite must email the MCTP to verify slot availabilities prior to consenting patients. Once a patient signs consent, the signed consent document and documentation of the consenting process must be faxed or securely emailed to the MCTP. The required forms, including Eligibility Criteria Checklist and Registration Form, can be found in the Supplemental Forms Document.

To register a subsite patient, the following documents must be completed by the subsite research team and faxed or securely e-mailed to the MCTP:

- Copy of all baseline tests required per the protocol calendar. Tests must be within the specified window.
- Signed Patient Consent Form, if not previously sent
- Signed Patient HIPAA Authorization Form (if separate), if not previously sent
- Consent Documentation Note, if not previously sent
- Completed & Signed Eligibility Checklist (refer to Supplemental Forms Document)
- Registration Form (refer to Supplemental Forms Document)
- Source documents verifying every inclusion & exclusion criteria

Upon receipt of registration documents, the MCTP will send an email confirming receipt. If confirmation of receipt is not received within 24 hours of submission, please contact the MCTP by phone and/or pager to confirm receipt.

Upon receipt of all required registration documents and upon verification the subsite patient meets all eligibility criteria, the MCTP will:

- Assign the patient a study sequence ID, if not already provided at time of consent
- Register the patient on the study
- Fax and/or e-mail the subsite the completed Registration Form with the assigned study sequence ID and registration date as confirmation of patient registration

Following registration, patients should begin protocol treatment within the time frame specified in the study calendar (Section 11.2). Issues that would cause treatment delays should be discussed with the Principal Investigator and MCTP as soon as possible. If a patient does not receive protocol therapy following registration, the PI and MCTP must be notified immediately within 1 business day.

Each participating institution will order study agents directly. Agents may be ordered by a participating site only after the required regulatory documents, including the initial IRB approval for the site, have been forwarded to the MCTP and all other study-specific requirements have been met (as outlined during site activation). Manufacturing materials are covered under a separate agreement.

6 TREATMENT PLAN

Because this is a cellular therapy clinical trial and the investigational intervention is a single event of CAR-T cell infusion (single dosing event for Cohort A, two infusions for Cohort B

and Cohort C), there is no plan for dose modification of CAR-T cells, cyclophosphamide or fludarabine after initiation. Patients who initially present with a calculated creatinine clearance less than 50 ml/min will receive fludarabine at a dose adjusted according to Section 7.2. Once fludarabine has been initiated at the adjusted dose, there will be no further modifications.

Reported adverse events and potential risks of CAR-T cells, cyclophosphamide and fludarabine are described in Section 8.0.

No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

6.1 Collection of peripheral blood mononuclear blood cells

Once the patient has provided consent and met eligibility criteria, he/she will undergo an apheresis procedure for collection of peripheral blood mononuclear cells for CAR-T manufacturing at the Apheresis Units of The Ohio State University or Nationwide Children's Hospital according to institutional standards.

6.2 Processing of peripheral blood mononuclear cells and manufacturing process to generate Anti-CD19/20/22 chimeric antigen receptor T (CAR-T) cells.

6.2.1 Processing and manufacturing facility

The site of processing of peripheral blood mononuclear collection products and subsequent Anti-CD19/20/22 CAR-T cell manufacture will be W.W. Williams Cellular Therapy Laboratory (CTL) located in the James Cancer Hospital, which is compliant with federal regulations and FACT standards for more than minimally manipulated cell products.

6.2.2 Anti-CD19/20/22 CAR-T cell manufacturing

Anti-CD19/20/22 CAR-T cells will be manufactured according to the processes described in the IND submission. After cell cultures are completed and release criteria specified in the IND are met, the CAR-T cells may be released for infusion to the patient or cryopreserved in infusible cryomedia.

6.3 Lymphodepletive chemotherapy

Lymphodepletive chemotherapy will be given to induce lymphopenia to facilitate engraftment and expansion of Anti-CD19/20/22 CAR-T cells.

- Cyclophosphamide 60 mg/kg (actual body weight [ABW], unless ABW > 125% of ideal body weight, then use 25% adjusted body weight) will be infused intravenously per institutional standard operating procedures on day -6.
- Fludarabine 25 mg/m^2 (ABW) will be infused intravenously daily over 30 minutes on days -5 to -3.

Pediatric lymphodepletive chemotherapy regimen is described in Appendix 7.

In cases when the CAR-T manufacture process is required to be extended from 6 to 12 days, as tested on day 5 onwards, it is possible that the chemotherapy day numbering will be modified accordingly.

Antiemetic premedications should be administered according to institutional standard operating procedures.

6.3.1 Therapy for symptom control or debulking prior to lymphodepletion

If treatment is required for control of disease – related symptoms or to decrease tumor – related bulk, patients may receive corticosteroids at a maximum daily dose of 200mg of prednisone or equivalent steroid dose (up to 7 days prior to infusion of CAR-T cells).

In patients with bulky disease (i.e. lymphoid mass ≥10cm or lymphocytosis >25,000/mcl), discussion with the principal investigator will be done to consider debulking therapy. If debulking therapy is to be used, agents with short half-lives may be used so that they will not be present at the time of infusion of CAR-T cells. Debulking would occur after lymphocyte collection and prior to lymphodepletive chemotherapy.

Note: In subjects who receive de bulking agent, the agent, dose, and date of administration will be documented as part of pre -lymphodepletion information. In addition, patients treated with de bulking agents will have disease – specific measurements of disease burden prior to starting lymphodepletion, including CT imaging (NHL, CLL, B-PLL, when applicable), bone marrow biopsy (ALL and CLL, B-PLL, NHL, Lymphoid blast crisis of CML when applicable and clinically indicated) and peripheral blood counts and peripheral blood flow cytometry as clinically indicated.

6.4 Study drug

6.4.1 Description

Anti-CD19/20/22 CAR-T cells are autologous T cells engineered to express an extracellular single chain antibody (scFv) with specificity for CD19, CD20 and CD22 linked to the intracellular signaling domains of the OX- 40 and TCRζ. Anti-CD19/20/22 CAR-T cells can be administered fresh or can be cryopreserved in infusible cryomedia prior to infusion. Human Anti-CD19/20/22 CAR-T cells will be administered in 1 or multiple bag(s). Bag aliquoting, cell content and cryomedia composition will be done according to specifications of the IND.

If manufacture of cells yields cell dose higher than the planned use, extra cell product will be cryopreserved for future research use related to persistence, phenotype, and efficacy profile. The informed consent reflects this sample storage and this research use, which also includes unused apheresis cells.

6.4.2 Preparation of Anti-CD19/20/22 CAR-T cells for infusion

The Anti-CD19/20/22 CAR-T cells are manufactured in the James laboratory as described in Section 6.2.1 and are not released from this facility until release criteria for the infused cells (e.g., cell purity, sterility, CAR-T cell dose, etc.) are met. Upon release, the cells are transported to the Stem Cell Transplant Unit of Arthur G. James Cancer Hospital or to Nationwide Children's Hospital. Cells being delivered to Nationwide Children's Hospital will be transported via an internal Nationwide Children's Hospital approved courier according to institutional practices. An example of the release form is attached in Appendix 3.

6.4.2.1 Second manufacturing procedure after a manufacturing failure

There is a potential for failure of the manufacturing process to yield a CAR-T cell product that meets criteria for infusion to the patient. If this occurs a second manufacture can be attempted. The following conditions should be met for a second collection to be considered:

- Agreement between treating physician and PI that trial participation continues to be in the best interest of the patient.
- Subjects will have to continue to meet eligibility criteria prior to the second collection.
- Subjects undergoing a second manufacture are not eligible for fresh product infusion.
- If patients receive additional therapy for their underlying malignancy prior to the second lymphocyte collection, all timelines related to preceding therapy should be followed.
- If patients do not receive additional therapy for their underlying malignancy prior to the second lymphocyte collection, but debulking therapy is considered to be administered prior to starting lymphodepletion, then all guidance regarding debulking therapy should be followed as delineated in Section 6.3.1.
- Second manufacturing attempt should occur no sooner than 2 weeks from the first attempt.

No more than 2 manufacturing attempts will be undertaken.

6.4.3 Cryopreserved vs. fresh product

This clinical trial is investigating the local manufacture of Anti-CD19/20/22 CAR-T cells for patients with lymphoid malignancies. Access to rapid manufacture of CAR-T cell product allows for rapid treatment with these agents of patients with rapidly progressive disease who would otherwise not have the option for treatment with other products with slower manufacture times.

The use of fresh product will require starting lymphodepletion while the product is manufacturing and frequent assessments of manufacturing success will be done through the process (i.e. cell counts on the manufacture product, projected CAR-T cell counts per kg of patient weight). Specifically, the product for the second infusion of the split dose for Cohort B and Cohort C patients will be frozen after harvest and prior to administration on Day 7. The option of cryopreservation of the product remains for patients who are not stable or for whom the stability of their disease permits waiting.

For patients whose clinical condition worsens after apheresis and before receipt of lymphodepleting chemotherapy, manufacturing of investigational product may continue followed by storage of cryopreserved product. Patients can be taken off study to allow receipt of bridging therapy for progressive disease or for management of infections. When the patient is eligible for lymphodepleting therapy, they may be re-enrolled on the study and may receive lymphodepleting chemotherapy followed by infusion of a cryopreserved product. In the event that the participant is not enrolled in the study, the cryopreserved product will be used for purposes of quality testing in the cell therapy lab.

In some instances, fresh infusion may not be feasible due to scheduling or change in medical status; For these patients who are planned to receive a cryopreserved product, safety check for receipt of lymphodepleting chemotherapy shall begin after the product has met release criteria and is already cryopreserved.

Freezing is allowed if clinical condition prevents administration of fresh product at the planned day. Delays of up to 7 days are allowed. If, for any reason, the patient cannot receive the first cell dose for more than 7 days, the subject will need to repeat lymphodepleting chemotherapy. Repeating lymphodepleting chemotherapy will be therefore allowed in this situation in 3-5 weeks from the last day of first lymphodepleting chemotherapy. **If patient is not able to receive lymphodepleting treatment in up to 5 weeks, he/she will be taken off study.**

6.4.4 Cell thawing (for frozen Anti-CD19/20/22 CAR-T cells)

If cells are frozen, they will be thawed at the bedside, one bag at a time, using a water bath maintained at 37°C (± 2°C). The bag will be gently massaged until the cells are thawed. If the Anti-CD19/20/22 CAR-T cell product appears to have a damaged or leaking bag or otherwise appears compromised, it should not be infused and should be returned to the manufacturing facility.

6.4.5 Premedication

It is recommended that subjects be premedicated with acetaminophen 650 mg by mouth and diphenhydramine hydrochloride 25-50 mg by mouth or IV, prior to the infusion of CAR- T cells. These medications may be repeated every six hours as needed. A course of non-steroidal anti-inflammatory medication may be prescribed if the patient continues to have fever not relieved by acetaminophen. **It is recommended that patients not receive systemic corticosteroids such as hydrocortisone, prednisone, prednisolone (Solu-Medrol) or dexamethasone (Decadron) at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on T cells.** If corticosteroids are required for an acute, life-threatening infusional reaction, an initial dose of hydrocortisone 100 mg is recommended.

Cell infusion guidelines for pediatric patients include premedication instructions and are described in in Appendix 7.

6.4.6 Administration

The CAR-T+ cells will be administered by rapid intravenous infusion per institutional standards. Fresh product will be infused according to institutional standards. Frozen product will be thawed at the bedside and infused per institutional standards. Each infusion bag will have affixed to it a label containing the following: "FOR AUTOLOGOUS USE ONLY." In addition, the label will have at least two unique identifiers such as the subject's initials, birth date, and study number. Prior to the infusion, two individuals will independently verify this information in the presence of the subject and confirm that the information is correctly matched to the participant.

Emergency medical equipment (i.e., emergency trolley) will be available during the infusion in case the subject has an allergic response, or severe hypotensive crisis, or any other reaction to the infusion. Vital signs (temperature, respiration rate, pulse, and blood pressure) will be taken before and after infusion, then every 15 minutes for at least one hour and until these signs are satisfactory and stable.

Cell infusion guidelines for pediatric patients are described in in Appendix 7.

Split – dose schedule for Cohort B and Cohort C patients

In order to minimize the risk of cytokine release syndrome (CRS) and immune effector cell associated neurotoxicity syndrome (ICANS), patients in Cohort B and Cohort C will have their dose split in two separate infusion dates.

- DAY 0: Infusion of fresh or frozen Anti-CD19/20/22 CAR-T cell product (40% of the total dose as assigned by the dose escalation schema), and
- DAY 7: Infusion of frozen Anti-CD19/20/22 CAR-T cell product (60% of the total dose as assigned by the dose escalation schema. Patients with any grade > 1 CRS or ICANS or unresolved grade = 1 CRS or ICANS will not receive this second infusion.

Note for dose # 2: 72 hour window from Day 7- Day 10 allowed to infuse cells if criteria are not met on Day 7.

6.5 Cytokine release syndrome

Cytokine release syndrome (CRS) is a potential risk of the infusion of CAR-T+ cells. The majority of cases of CRS will occur within the first 48-72 hours of infusion, but can occur later.[46] Based on tumor volume and clinical presentation, prophylactic tocilizumab may be administered at the discretion of the investigator.

Signs and symptoms associated with CRS include

- Constitutional: Fever ± rigors, malaise, fatigue, anorexia, myalgias, arthralgias, nausea, vomiting, headache;
- Skin: Rash;
- Gastrointestinal: Nausea, vomiting, diarrhea;
- Respiratory: Tachypnea, hypoxemia;

- Cardiovascular: Tachycardia, widened pulse pressure, hypotension, increased, cardiac output (early), potentially diminished cardiac output (late);
- Coagulation: Elevated D-dimer, hypofibrinogenemia, bleeding;
- Renal: Azotemia;
- Hepatic: Transaminitis, hyperbilirubinemia;
- Neurologic: Headache, mental status changes, confusion, delirium, word finding difficulty or frank aphasia, hallucinations, tremor, dysmetria, altered gait, seizures.

In this study, the signs and symptoms of CRS outlined above will be graded according to the recent revision of CRS criteria presented in the ASBMT (now ASTCT) Consensus Grading for Cytokine Release Syndrome and Neurologic Toxicity Associated with Immune Effector Cells.[46,47,58] This consensus has provided an updated grading system for CRS (Table 6.5).

Table 6.5: ASBMT CRS Consensus Grading				
	Grade 1	Grade 2	Grade 3	Grade 4
Fever*	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C	Temperature ≥38°C
	With			
Hypotension[1]†	None	Not requiring vasopressors	Requiring a vasopressor with or without vasopressin	Requiring multiple vasopressors (excluding vasopressin)
	and/or			
Hypoxia†	None	Requiring low-flow nasal	Requiring high-flow nasal	Requiring positive
		cannula† or blow-by	cannula‡, facemask, nonrebreather mask, or Venturi mask	pressure (e.g., 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

[1]Hypotension defined by:

- Age 1 to 10 years: SBP less than [70 + (2x age in years)] mm Hg
- Age greater than 10 years: SBP less than 90 mmHg

6.5.1 CRS management algorithm

Management of CRS will be according to institutional SOP for management of adverse events following immune effector cell therapy (Appendix 4). This management plan is summarized in the algorithm delineated in Figure 6.5.

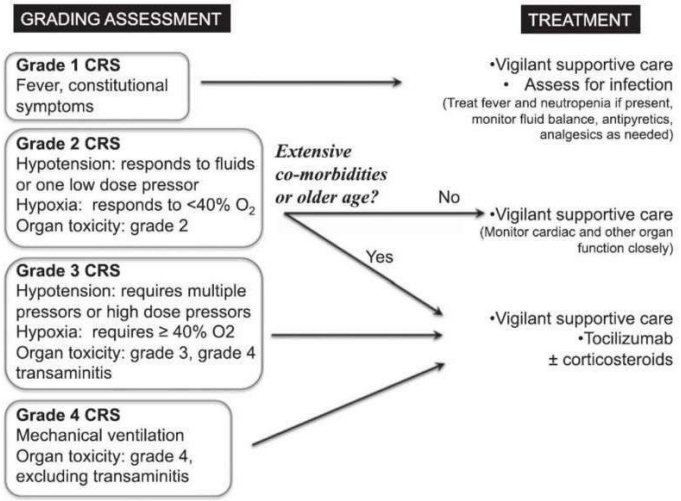

Figure 6.5: CRS treatment algorithm[47]

No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the subject's malignancy.

Cytokine release syndrome in pediatric patients should be treated according to Nationwide Children's Hospital clinical practice guidelines for CAR T cell related toxicities.

6.6 Neurotoxicity (Immune effector cell–associated neurotoxicity syndrome [ICANS])

Patients treated in previous clinical trials of anti-CD19 CAR-T cells have presented varying degrees of neurotoxicity.[5,6] Neelapu and colleagues reported a median onset to neurologic symptoms of 5 days, with 28% presenting grade 3 and higher toxicity.[5] Early events included dysphasia, inattention, calculation defects and handwriting difficulty. Most common events included encephalopathy (21%) aphasia (9%) and confusion (9%).

ICANS events are graded based on the ASBMT consensus grading for adults (Table 6.6-1) or children (Table 6.6-3).

Table 6.6-1: ASBMT ICANS Consensus Grading for Adults

Neurotoxicity score	Grade 1	Grade 2	Grade 3	Grade 4
ICE (immune effector cell-associated encephalopathy) score*	7 – 9	3 – 6	0 – 2	0 (patient is unarousable and unable to perform ICE)

Depressed level of consciousness†	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma.
Seizure	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly or nonconvulsive seizures on EEG that resolve with intervention	Life – threatening prolonged seizure (>5 min); or repetitive clinical or electrical seizures without returning to baseline in between.
Motor findings‡	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis
Elevated ICP/ cerebral edema	N/A	N/A	Focal / local edema on neuroimaging€	Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad.

ICANS grade is determined by the most severe event (ICE score, level of consciousness, seizure, motor findings, raised ICP/cerebral edema) not attributable to any other cause;
* 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 (e.g. no sedating medication).
‡ Tremors and myoclonus associated with immune effector cell therapies may be grade 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

The ICE tool is presented in Table 6.6-2.

Table 6.6-2: ICE tool for encephalopathy assessment

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

Table 6.6-3: ASBMT ICANS Consensus Grading for Children

Neurotoxicity domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE score for children ≥ 12 years*	7 – 9	3 – 6	0 – 2	0 (patient is unarousable and unable to perform ICE)

CAPD (Cornell Assessment of Pediatric Delirium) 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	Patient is unarousable or requires vigorous or repetitive tactile simuli 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 returning to baseline in between
Motor findings (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€	Diffuse cerebral edema on neuroimaging; decerebrate or decorticate posturing; or cranial nerve VI palsy; or papilledema; or Cushing's triad.

ICANS grade is determined by the most severe event (ICE/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 grade 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

Table 6.6-4 delineates the encephalopathy assessment for children ≤12 years of age.

Table 6.6-4: CAPD assessment of pediatric encephalopathy

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 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?					

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 (i.e. blanket or stuffed animal);
4. Uses single words or signs;
5. No sustained calm state;
6. Not soothed by usual comforting actions, e.g. Singing, holding, talking and reading;
7. Little if any play, efforts to sit up, and if mobile crawl or walk around
8. Not following simple directions. If verbal, not engaging in simple dialog with words or jargon.

Management of Neurologic Toxicities:

- Subjects should be monitored daily for the first 7 days. Assessment with the ICE/CAPD tool should be done daily (Table 6.6-2 and Table 6.6-4).
- Early events include changes in hand-writing, calculation defects, dysphasia and inattention.
- If neurologic symptoms are present, consider ICANS, consider other possible causes.
- If Grade ≥2 or higher neurologic toxicities, subjects should be monitored with continuous cardiac telemetry and pulse oximetry.
- Subjects with severe (Grade ≥3) neurologic symptoms should be considered for transfer to intensive care unit.
- Non – sedating, anti-seizure medications (i.e. levetiracetam) should be considered for any Grade ≥2 neurologic toxicity.

Management guidelines are present in Table 6.6-5. Additional management guidelines as per the Arthur G. James Cancer Hospital Standard Operating Procedure Management of Patients Receiving Immune Effector Cell Therapy Appendix 4 .

Table 6.6-5: General management guidelines Immune effector cell – associated neurotoxicity syndrome [ICANS]		
Neurologic event (ASBMT or CTCAE v5.0 Grade when ASBMT not applicable)	Concurrent CRS	No concurrent CRS
Grade 1	Manage per CRS guideline	Monitor neurologic symptoms daily
Grade 2 • Examples: Somnolence 0 moderate, limiting instrumental ADLs Moderate confusion Encephalopathy limiting instrumental ADLs Dysphasia Seizures	• Tocilizumab per CRS guideline • If no improvement within 24 hours of tocilizumab, administer dexamethasone 10mg intravenously every 6 hours (if no other steroids are started). • Continue dexamethasone until event grade ≤1 and then taper over 3 days. • Consider non-sedating antiseizure medications.	• Dexamethasone, 10mg intravenously every 6 hours. • Continue dexamethasone until event grade ≤1 and then taper over 3 days. • Consider non-sedating antiseizure medications.
• Grade 3 Examples: Somnolence, confusion, severe disorientation, encephalopathy, severe dysphasia.	• Tocilizumab per CRS guidelines • Methylprednisolone 1000mg intravenously daily (at same time as tocilizumab); continue for 2 additional days, if improves, change to dexamethasone and manage as grade 2. • Consider non-sedating antiseizure medications.	• Methylprednisolone 1000mg intravenously daily for 3 days, if improves, then change to dexamethasone and manage as grade 2. • Consider non-sedating antiseizure medications.

Grade 4 Life threatening consequences. Urgent intervention needed. Requirement for mechanical ventilation. Rule out cerebral edema.	• Tocilizumab per CRS guidelines • Methylprednisolone 1000mg intravenously daily (at same time as tocilizumab); continue for 2 additional days, if improves, change to dexamethasone and manage as grade 2. • Consider non-sedating antiseizure medications.	• Methylprednisolone 1000mg intravenously daily for 3 days, if improves, then change to dexamethasone and manage as grade 2 and 3. • Consider non-sedating antiseizure medications.

Table 6.6-6: James Cancer Center SOP for Management of Neurologic Toxicity for CAR T and other Immune Effector Cell Therapy

Grade	Treatment Recommendation
1	• Levetiracetam (Keppra®) prophylaxis (500 mg PO/IV q 12 hours). • Consider neurology consult.
2	• Levetiracetam (Keppra®) prophylaxis (as above) • Brain imaging (MRI preferred over CT scan). • EEG monitoring. • Neurology Consult. • Consider Diagnostic Lumbar Puncture (unless clinically contraindicated). • Consider transfer to MICU (in particular if CRS grade ≥2). • **Steroid therapy:*** o Dexamethasone 10 mg IV x1 dose. And if persistent grade 2, then o Dexamethasone 10 mg IV every 6 hours, then taper.[1] o If no improvement or progression in 48-72 hours, consider therapies listed for grac 4.
3	• Levetiracetam (Keppra®) prophylaxis (as above), or therapy (if applicable). • Brain imaging (MRI preferred), and may repeat every 2-3 days if persistent grade ≥ toxicity. • EEG monitoring. • Neurology Consult. • Diagnostic Lumbar Puncture (unless clinically contraindicated). • Transfer to MICU is recommended. • **Steroid therapy:*** o Dexamethasone 10 mg IV every 6 hours, then taper.[1] o If no improvement or progression in 48-72 hours consider therapies listed for grac 4.
4	• Levetiracetam (Keppra®) prophylaxis (as above), or therapy (if applicable).

	• Brain imaging (MRI preferred), and may repeat every 2-3 days if persistent grade ≥ toxicity.
	• EEG monitoring.
	• Neurology Consult.
	• Diagnostic Lumbar Puncture (unless clinically contraindicated).
	• Transfer to MICU (consider mechanical ventilation for airway protection).
	• **Steroid therapy:**[1]
	  ○ Solumedrol 1 gm IV daily **(for at least 3 days)** until neurotoxicity is ≤ G1, the taper.[1]
	• If grade 4 neurotoxicity persists x ≥72 hours, consider the following:
	  ○ LP with IT triple therapy (MTX 15 mg, Cytarabine 40 mg and Hydrocortisone 50 mg).
	  ○ Siltuximab 11mg/kg x 1 dose, may repeat in 10 days.
	  ○ Anakinra.
	  ○ ATG (may eliminate CAR-T cells).

[1]Steroid taper will follow the same guidelines as with the CRS (see before).

Neurologic toxicity in pediatric patients should be treated according to Nationwide Children's Hospital clinical practice guidelines for CAR T cell related toxicities.

Hemophagocytic lymphohistiocytosis (HLH)/ Macrophage activation syndrome (MAS).[58]

HLH/MAS should be suspected if he/she had a peak serum ferritin level of >10,000ng/ ml during the cytokine-release syndrome phase of CAR-T-cell therapy (typically the first 5 days after cell infusion) and subsequently developed any two of the following (grades as per CTCAE Version 4):

• Grade ≥3 increase in serum bilirubin, aspartate aminotransferase, or alanine aminotransferase levels
• Grade ≥3 oliguria or increase in serum creatinine levels
• Grade ≥3 pulmonary edema
• Presence of hemophagocytosis in bone marrow or organs based on histopathological assessment of cell morphology and/or CD68 immunohistochemistry

Monitoring: Established cases will have daily monitoring of blood ferritin, LDH, fibrinogen, transaminases, bilirubin, and serum creatinine levels until resolved or stabilized.

Plan of Care: per Institutional SOP.

- Grade ≥ 3 organ toxicity will be managed with tocilizumab (per CRS algorithm) plus steroids (e.g. Dexamethasone 10 mg IV every 6 hours and tapered as per CRS guidelines). If no improvement after 48 hours, consider the following:
- Intrathecal cytarabine (100 mg), and hydrocortisone (50-100 mg) if associated neurotoxicity.
- Anakinra (second line after steroid failure) with or without steroid escalation to Methylprednisolone 1 gm daily (then taper as per CRS guidelines).
- Etoposide 75-100 mg/m2 IV and it may be repeated after 4-7 days (if clinically indicated). This may be used ONLY after discussion with the primary physician who prescribed the CAR T therapy. Etoposide therapy will likely eliminate the

CAR T cells, thus reserved for life-threatening situations after failure of both anakinra and steroid.

Toxicity in pediatric patients should be treated according to Nationwide Children's Hospital clinical practice guidelines for CAR T cell related toxicities.

Other expected toxicities include tumor lysis syndrome, seizures, atypical hemolytic uremic syndrome, opportunistic infections, coagulopathy and cytopenias.

6.7 Phase I Dose Escalation

Dose escalation will proceed within each cohort according to the following schema. Dose-limiting toxicity (DLT) is defined in Section 6.8.

Table 6.7: Dose Escalation Schema.

Number of Subjects with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 subjects at the next dose level.
1 out of 3	Enter 3 more subjects at this dose level. ● If 0 of these 3 subjects experience DLT, proceed to the next dose level. ● If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional subjects will be entered at the next lowest dose level if only 3 subjects were treated previously at that dose. In case this situation occurs at dose level 1, subsequent subjects will be dosed at level -1.
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional subjects will be
	entered at the next lowest dose level if only 3 subjects were treated previously at that dose. In case this situation occurs at dose level 1, subsequent subjects will be dosed at level -1.
$\leq$1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended maximally tolerated dose. At least 6 subjects should be entered at the recommended phase 2 dose.

6.8 Definition of Dose-Limiting Toxicity

Management of common toxicities and dose modifications are outlined in Section 7.0.

In this study, a dose limiting toxicity (DLT) includes those adverse events graded according to NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0, with the

exception of cytokine release syndrome to be graded using criteria provided in Section 6.4. DLT definitions also apply to dose level -1. To be DLTs, adverse events must be suspected to be secondary to CAR-T cell infusion, occur during the first 30 days after infusion and meet the following criteria:

1. Cytokine release syndrome (CRS) related toxicities
    a. Grade 4 CRS of any duration.
    b. Toxicities associated with grade 3 cytokine release syndrome that do not improve to grade ≤ 2 within 72 hours of intervention;
2. Grade 3 or 4 immune effector cell-associated neurotoxicity syndrome of any duration
3. Any grade 4 life-threatening toxicity.
4. Any grade 3 non hematologic toxicity involving a vital organ system, with the following exceptions
    - Laboratory abnormalities without associated symptomatology or clinical consequence that resolve in less than 7 days;
    - Laboratory abnormalities compatible with tumor lysis syndrome.

6.9 General Concomitant Medications and Supportive Care Guidelines

Subjects should receive full supportive care, including transfusions of blood and blood products, cytokines, antibiotics, antiemetics, etc. when appropriate.

6.10 Criteria for removal from treatment

Treatment and follow up in this protocol may continue until one of the following criteria applies:

- Disease progression, treatment failure or recurrence at any time:
    - Patients will continue with safety, survival and correlative follow up. Correlative blood samples will be collected when feasible; if bone marrow or lymph node biopsies are planned as standard of care, correlative collection is optional. Only SAEs related to study treatment will be recorded through month 12.
- Intercurrent illness that prevents infusion or follow up post-infusion;
- Pregnancy during the course of the study for a child-bearing participant
- The investigator considers it, for safety reasons, to be in the best interest of the patient;
- General or specific changes in the patient's condition render the patient unacceptable for treatment (before infusion) or follow-up (after infusion) in the judgment of the investigator.

6.11 Criteria for Removal from Study

Treatment and follow up in this protocol may continue until one of the following criteria applies:

- Patient proceeds on another clinical trial before receiving the CART cells
- Patient decision to withdraw prior to infusion or from post-infusion follow-up
- Death

The Sponsor reserves the right to temporarily suspend or prematurely discontinue this study. The date and reason for discontinuation must be documented. Every effort should be made to complete the appropriate assessments.

For subjects who withdraw consent, there must be clear documentation of whether the subject withdraws consent to treatment only (i.e. agrees to follow-up) or withdraws consent to treatment and follow-up.

6.12 Duration of Follow Up

Subjects will be followed for acute toxicity for 30 days after treatment has been discontinued or until death, whichever occurs first. From day 30 through 12 months, patients will continue to be monitored for disease assessment and safety.

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation will be recorded and reported immediately.

Subjects will be followed for persistence of the provirus in peripheral blood at 0, 7, 14, 21, 30, 60, 90 days, 6 and 12 months, and annually thereafter until vector sequences are no longer detectable by qPCR. Subjects will also be followed for replication competent lentivirus (RCL) using the VSV-G assay qPCR assay at 3, 6, and 12 months and yearly thereafter. If RCL is not detected at 1 year, follow-up will be discontinued.

Follow up will be 15 years for monitoring for potential gene therapy–related delayed adverse events.

During the first 5 years after treatment with Anti-CD19/20/22 CAR-T cells, subject follow up will be done annually in clinical visits, in person, following the form delineated in Appendix 5, which will record the following information:

- Exposure to mutagenic agents;
- Exposure to medicinal products;
- History;
- Physical examination;
- Laboratory test results including assay for persistent vector sequence;
- Emergence of new conditions:
    - New malignancy(ies)
    - New incidence or exacerbation of a pre-existing neurologic disorder

- New incidence or exacerbation of a pre-existing rheumatologic or autoimmune disorder
- New incidence of a hematologic disorder.

During the subsequent 10 years (years 6 – 15 after treatment with Anti-CD19/20/22 CAR-T cells), subjects will be contacted annually. If office visits are not scheduled, then patients may be contacted by telephone or written questionnaire. Contact throughout years 6 – 15 will be done following the form in Appendix 6, which will record the following information:

- Emergence of new conditions, including but not limited to:
  - New malignancy(ies)
  - New incidence or exacerbation of a pre-existing neurologic disorder
  - New incidence or exacerbation of a pre-existing rheumatologic or autoimmune disorder
  - New incidence of a hematologic disorder.

During years 6 – 15 after treatment, subjects with previous laboratory results indicating vector persistence will have this test repeated annually until resolution.

All subjects will be provided with the study coordinator contact information and encouraged to report delayed adverse events, including unexpected illness or hospitalization.

6.13 Retreatment: Not permitted.

Re-treatment with Anti-CD19/20/22 CAR-T cells is not permitted under the current protocol.

6.14 Stopping Rules

In the event of any of the following, study enrollment will be suspended pending safety review and recommendations by the DSMC.

- Death from any cause other than disease progression or events clearly unrelated to the study product within 30 days of infusion.
- Diagnosis of a malignancy of T-cell origin in any subject who has received Anti-CD19/20/22 CAR T cells, until insertional mutagenesis is ruled out
- Occurrence in two or more subjects of any Grade 4 dose-limiting toxicities
- Product manufacturing failures: A safety review will be done after 5 products have been manufactured. A manufacturing failure rate of up to 20% can be expected in subjects with ALL, CLL, or B-PLL, whereas NHL subjects are expected to have up to 10% product manufacturing failure. For this study, manufacturing failure in excess of 20% will require trial stopping and review of the product characteristics, underlying diagnoses associated with failure. In addition, if prior to reaching 5 manufacturing events, 1 or more subjects have a manufacturing failure, the trial will be stopped and re-initiation plans will be submitted to the Food and Drug Administration.

6.15 Prohibited medications

Treatment with Anti-CD19/20/22 CAR-T cell depends on the collection and persistence of functional infused cells. Because of this, medications that may be lymphotoxic should be avoided in certain periods (**unless required for management of the underlying disease or toxicities of the Anti-CD19/20/22 CAR-T**). Agents to avoid include:

- Corticosteroids;
- Lymphodepletive agents including alemtuzumab and antithymocyte globulin;
- Immunosuppressants.

These agents should be avoided 10 days prior to peripheral blood collection, 3 days prior to Anti-CD19/20/22 CAR-T cell infusion and for 90 days after infusion.

7 DOSE DELAYS/DOSE MODIFICATIONS

7.1 Cyclophosphamide

There are no planned dose modifications for cyclophosphamide.

7.2 Fludarabine

Fludarabine dose will be modified for patients who present a creatinine clearance (CrCl) of less than 50ml/min, calculated by the Cockcroft – Gault formula.

- If Creatinine Clearance is >50ml/ml, then no fludarabine dose adjustment is necessary.
- If Creatinine Clearance is between 30 and 50ml/min, then fludarabine should be given at 75% of the calculated dose (25% dose reduction), on days -5 to -3.
- If Creatinine is less than 30ml/min, then use of fludarabine is contraindicated and the patient will be excluded from the trial.

Once fludarabine treatment has been initiated as adjusted above, there will be no further dose modifications.

7.3 Anti-CD19/20/22 CAR-T cells

There are no planned dose modifications for the CAR-T cell infusion.

8 ADVERSE EVENTS AND POTENTIAL RISKS

The following is a list of AEs (Section 8.1) and the reporting requirements associated with observed AEs (Section 8.3 and Section 8.4).

The clinical course of each event will be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause.

Serious adverse events that are still ongoing at the end of the study period will necessitate follow-up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study

participation will be recorded and reported immediately (within approximately 24 hours of acknowledgement).

Please refer to the package insert(s) for the comprehensive list of adverse events.

8.1 Adverse Events

8.1.1 Chimeric Antigen Receptor T (CAR-T) cells

Anti-CD19 CAR-T cells have presented two main toxicities: cytokine release syndrome and neurotoxicity.

Cytokine release syndrome (CRS) occurs usually in the first 2 weeks after CAR-T cell infusion and is manifested by the following spectrum of findings:

- fever;
- malaise and constitutional symptoms;
- hypotension;
- capillary leak, edema, pleural effusion or pulmonary congestion;
- hypoxemia;
- coagulopathy secondary to disseminated intravascular coagulation;
- end organ dysfunction, including respiratory failure, cardiovascular impairment, renal insufficiency;

Management of cytokine release syndrome includes early detection through frequent monitoring, intensive supportive care, including intensive care unit admission, monitoring of C reactive protein throughout the course of the reaction, and administration of tocilizumab, a humanized monoclonal antibody directed against interleukin 6 receptor, a cytokine that plays a central role in inflammatory and immune reactions.

Rarely, severe CRS can progress to called hemophagocytic lymphohistiocytosis (HLH), an inflammatory condition triggered by very high levels of cytokines.

Neurotoxicity occurs independently of CRS, although usually at a slightly later time point. The pathogenesis of neurotoxicity is not well understood. The manifestations include:

- delirium,
- speech disturbances;
- focal neurologic deficits;
- fine motor impairment;
- seizures;
- coma (rare).

The management of neurotoxicity includes frequent monitoring of the neurologic status of the patient and their fine motor skills; once sustained or ≥ Grade 2 neurotoxicity is observed, corticosteroids (primarily dexamethasone) are used for treatment of this adverse event.

Although rare, there are descriptions of cases of cerebral edema associated with CAR-T

infusions. This latter reaction is idiosyncratic and there are no predictive parameters identified so far.

8.1.2 Fludarabine

The most common adverse events include myelosuppression (neutropenia, thrombocytopenia and anemia), fever and chills, infection, and nausea and vomiting. Other commonly reported events include malaise, fatigue, anorexia, and weakness. Serious opportunistic infections have occurred in CLL patients treated with fludarabine. Adverse events and those reactions that are more clearly related to the drug are arranged below according to body system.

Hematopoietic Systems
Hematologic events (neutropenia, thrombocytopenia, and/or anemia) were reported in the majority of CLL patients treated with fludarabine. During fludarabine treatment of 133 patients with CLL, the absolute neutrophil count decreased to less than 500/mm3 in 59% of patients, hemoglobin decreased from pretreatment values by at least 2 grams percent in 60%, and platelet count decreased from pretreatment values by at least 50% in 55%. Myelosuppression may be severe, cumulative, and may affect multiple cell lines. Bone marrow fibrosis occurred in one CLL patient treated with fludarabine.

Several instances of trilineage bone marrow hypoplasia or aplasia resulting in pancytopenia, sometimes resulting in death, have been reported in postmarketing surveillance. The duration of clinically significant cytopenia in the reported cases has ranged from approximately 2 months to approximately 1 year. These episodes have occurred both in previously treated or untreated patients.

Life-threatening and sometimes fatal autoimmune phenomena such as hemolytic anemia, autoimmune thrombocytopenia/ thrombocytopenic purpura (ITP), Evans syndrome, and acquired hemophilia have been reported to occur in patients receiving fludarabine. The majority of patients rechallenged with fludarabine developed a recurrence in the hemolytic process.

In postmarketing experience, cases of myelodysplastic syndrome and acute myeloid leukemia, mainly associated with prior, concomitant or subsequent treatment with alkylating agents, topoisomerase inhibitors, or irradiation have been reported.

Infections
Serious and sometimes fatal infections, including opportunistic infections and reactivations of latent viral infections such as VZV (Herpes zoster), Epstein-Barr virus and JC virus (progressive multifocal leukoencephalopathy) have been reported in patients treated with fludarabine.

Rare cases of Epstein Barr Virus (EBV) associated lymphoproliferative disorders have been reported in patients treated with fludarabine.

Metabolic
Tumor lysis syndrome has been reported in CLL patients treated with fludarabine. This complication may include hyperuricemia, hyperphosphatemia , hypocalcemia, metabolic acidosis, hyperkalemia, hematuria, urate crystalluria, and renal failure. The onset of this syndrome may be heralded by flank pain and hematuria.

Nervous System
Objective weakness, agitation, confusion, seizures, visual disturbances, optic neuritis, optic neuropathy, blindness and coma have occurred in CLL patients treated with fludarabine at the recommended dose. Peripheral neuropathy has been observed in patients treated with fludarabine and one case of wrist-drop was reported.

In postmarketing experience, cases of progressive multifocal leukoencephalopathy have been reported. Most cases had a fatal outcome. Many of these cases were confounded by prior and/or concurrent chemotherapy. The time to onset has ranged from a few weeks to approximately one year after initiating treatment.

Impairment of Fertility
Studies in mice, rats and dogs have demonstrated dose-related adverse effects on the male reproductive system. Observations consisted of a decrease in mean testicular weights in mice and rats with a trend toward decreased testicular weights in dogs and degeneration and necrosis of spermatogenic epithelium of the testes in mice, rats and dogs. The possible adverse effects on fertility in humans have not been adequately evaluated.

Pulmonary System
Pneumonia, a frequent manifestation of infection in CLL patients, occurred in 16%, and 22% of those treated with fludarabine in the MDAH and SWOG studies, respectively. Pulmonary hypersensitivity reactions to fludarabine characterized by dyspnea, cough and interstitial pulmonary infiltrate have been observed.

In postmarketing experience, cases of severe pulmonary toxicity have been observed with fludarabine use, which resulted in ARDS, respiratory distress, pulmonary hemorrhage, pulmonary fibrosis, and respiratory failure. After an infectious origin has been exclude d, some patients experienced symptom improvement with corticosteroids.

Gastrointestinal System
Gastrointestinal disturbances such as nausea and vomiting, anorexia, diarrhea, stomatitis and gastrointestinal bleeding have been reported in patients treated with fludarabine.

Cardiovascular
Edema has been frequently reported. One patient developed a pericardial effusion possibly related to treatment with fludarabine. No other severe cardiovascular events were considered to be drug related.

Genitourinary System

Rare cases of hemorrhagic cystitis have been reported in patients treated with fludarabine.

Skin
Skin toxicity, consisting primarily of skin rashes, has been reported in patients treated with fludarabine.

Erythema multiforme, Stevens-Johnson syndrome, toxic epidermal necrolysis, and pemphigus have been reported, with fatal outcomes in some cases. Worsening or flare up of pre-existing skin cancer lesions, as well as new onset of skin cancer, has been reported in patients during or after treatment with fludarabine.

Data in the following table are derived from the 133 patients with CLL who received fludarabine in the MDAH and SWOG studies:

Table 8.1: Adverse events of fludarabine

Adverse Event	MDAH (n = 101) (%)	SWOG (n = 32) (%)
Any adverse event	88	92
Body as a whole	72	84
Fever	60	69
Chills	11	19
Fatigue	10	38
Infection	33	44
Pain	20	22
Malaise	8	6
Diaphoresis	1	13
Alopecia	0	3
Anaphylaxis	1	0
Hemorrhage	1	0
Hyperglycemia	1	6
Dehydration	1	0
Neurological	21	69
Weakness	9	65
Paresthesia	4	12
Headache	3	0
Visual disturbance	3	15

Hearing loss	2	6
Sleep disorder	1	3
Depression	1	0
Cerebellar syndrome	1	0
Impaired mentation	1	0
Pulmonary	35	69
Cough	10	44
Pneumonia	16	22
Dyspnea	9	22
Sinusitis	5	0
Pharyngitis	0	9
Upper respiratory infection	2	16
Allergic pneumonitis	0	6
Epistaxis	1	0
Hemoptysis	1	6
Bronchitis	1	0
Hypoxia	1	0
Gastrointestinal	46	63
Nausea/vomiting	36	31
Diarrhea	15	13
Anorexia	7	34
Stomatitis	9	0
Gastrointestinal bleeding	3	13
Esophagitis	3	0
Mucositis	2	0
Liver failure	1	0
Abnormal liver function tests	1	3
Cholelithiasis	0	3
Constipation	1	3
Dysphagia	1	0
Cutaneous	17	18

Rash	15	15
Pruritus	1	3
Seborrhea	1	0
Genitourinary	12	22
Dysuria	4	2
Urinary infections	2	15
Hematuria	2	3
Renal failure	1	0
Abnormal renal function tests	1	0
Proteinuria	1	0
Hesitancy	0	3
Cardiovascular	12	38
Edema	8	19
Angina	0	6
Congestive heart failure	0	3
Arrhythmia	0	3
Supraventricular tachycardia	0	3
Myocardial infarction	0	3
Deep venous thrombosis	1	3
Phlebitis	1	3
Transient ischemic attack	1	0
Aneurysm	1	0
Cerebrovascular accident	0	3
Musculoskeletal	7	16
Myalgia	4	16
Osteoporosis	2	0
Arthralgia	1	0
Tumor lysis syndrome	1	0

Thousands of adult patients received fludarabine in studies of other leukemias, lymphomas, and other solid tumors. The spectrum of adverse effects reported in these studies was consistent with the data presented above.

8.1.3 Cyclophosphamide

Hematopoietic system: Neutropenia occurs in patients treated with cyclophosphamide. The degree of neutropenia is particularly important because it correlates with a reduction in resistance to infections. Fever without documented infection has been reported in neutropenic patients.

Gastrointestinal system: Nausea and vomiting occur with cyclophosphamide therapy. Anorexia and, less frequently, abdominal discomfort or pain and diarrhea may occur. There are isolated reports of hemorrhagic colitis, oral mucosal ulceration and jaundice occurring during therapy.

Skin and its structures: Alopecia occurs in patients treated with cyclophosphamide. Skin rash occurs occasionally in patients receiving the drug. Pigmentation of the skin and changes in nails can occur.

The following adverse reactions have been identified from clinical trials or post-marketing surveillance. Because they are reported from a population from unknown size, precise estimates of frequency cannot be made.

Cardiac: cardiac arrest, ventricular fibrillation, ventricular tachycardia, cardiogenic shock, pericardial effusion (progressing to cardiac tamponade), myocardial hemorrhage, myocardial infarction, cardiac failure (including fatal outcomes), cardiomyopa thy, myocarditis, pericarditis, carditis, atrial fibrillation, supraventricular arrhythmia, ventricular arrhythmia, bradycardia, tachycardia, palpitations, QT prolongation.

Post-Marketing Experience:

Congenital, Familial and Genetic: intra-uterine death, fetal malformation, fetal growth retardation, fetal toxicity (including myelosuppression, gastroenteritis).

Ear and Labyrinth: deafness, hearing impaired, tinnitus. Endocrine: water intoxication. Eye: visual impairment, conjunctivitis, lacrimation.

Gastrointestinal: gastrointestinal hemorrhage, acute pancreatitis, colitis, enteritis, cecitis, stomatitis, constipation, parotid gland inflammation.

General Disorders and Administration Site Conditions : multiorgan failure, general physic al deterioration, influenza-like illness, injection/infusion site reactions (thrombosis, necrosis, phlebitis, inflammation, pain, swelling, erythema), pyrexia, edema, chest pain, mucosa l inflammation, asthenia, pain, chills, fatigue, malaise, headache.

Hematologic: myelosuppression, bone marrow failure, disseminated intravascular coagulation and hemolytic uremic syndrome (with thrombotic microangiopathy).

Hepatic: veno-occlusive liver disease, cholestatic hepatitis, cytolytic hepatitis, hepatitis,

cholestasis; hepatotoxicity with hepatic failure, hepatic encephalopathy, ascites, hepatomegaly, blood bilirubin increased, hepatic function abnormal, hepatic enzymes increased.

Immune: immunosuppression, anaphylactic shock and hypersensitivity reaction.

Infections: The following manifestations have been associated with myelosuppression and immunosuppression caused by cyclophosphamide: increased risk for and severity of pneumonias (including fatal outcomes), other bacterial, fungal, viral, protozoal and, parasitic infections; reactivation of latent infections, (including viral hepatitis, tuberculosis), Pneumocystis jiroveci, herpes zoster, Strongyloides, sepsis and septic shock.

Investigations: blood lactate dehydrogenase increased, C-reactive protein increased.

Metabolism and Nutrition: hyponatremia, fluid retention, blood glucose increased, blood glucose decreased.

Musculoskeletal and Connective Tissue: rhabdomyolysis, scleroderma, muscle spasms, myalgia, arthralgia.

Neoplasms: acute leukemia, myelodysplastic syndrome, lymphoma, sarcomas, renal cell carcinoma, renal pelvis cancer, bladder cancer, ureteric cancer, thyroid cancer.

Nervous System: encephalopathy, convulsion, dizziness, neurotoxicity has been reported and manifested as reversible posterior leukoencephalopathy syndrome, myelopathy, peripheral neuropathy, polyneuropathy, neuralgia, dysesthesia, hypoesthesia, paresthesia, tremor, dysgeusia, hypogeusia, parosmia.

Pregnancy: premature labor. Psychiatric: confusional state.

Renal and Urinary: renal failure, renal tubular disorder, renal impairment, nephropathy toxic, hemorrhagic cystitis, bladder necrosis, cystitis ulcerative, bladder contracture, hematuria, nephrogenic diabetes insipidus, atypical urinary bladder epithelial cells.

Reproductive System: infertility, ovarian failure, ovarian disorder, amenorrhea, oligomenorrhea, testicular atrophy, azoospermia, oligospermia.

Respiratory: pulmonary veno-occlusive disease, acute respiratory distress syndrome , interstitial lung disease as manifested by respiratory failure (including fatal outcomes), obliterative bronchiolitis, organizing pneumonia, alveolitis allergic, pneumonitis , pulmonary hemorrhage; respiratory distress, pulmonary hypertension, pulmonary edema, pleural effusion, bronchospasm, dyspnea, hypoxia, cough, nasal congestion, nasal discomfort, oropharyngeal pain, rhinorrhea.

Skin and Subcutaneous Tissue: toxic epidermal necrolysis, Stevens-Johnson syndrome,

erythema multiforme, palmar-plantar erythrodysesthesia syndrome, radiation recall dermatitis, toxic skin eruption, urticaria, dermatitis, blister, pruritus, erythema, nail disorder, facial swelling, hyperhidrosis.

Tumor lysis syndrome: like other cytotoxic drugs, cyclophosphamide may induce tumor-lysis syndrome and hyperuricemia in patients with rapidly growing tumors.

Vascular: pulmonary embolism, venous thrombosis, vasculitis, peripheral ischemia, hypertension, hypotension, flushing, hot flush.

8.2 Definitions

8.2.1 Adverse Event

An **adverse event** (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. The event can include abnormal laboratory findings, symptoms, or disease associated with the research study. The event does not necessarily have to have a causal relationship with the research, any risk associated with the research, the research intervention, or the research assessments.

Adverse events may be the result of the interventions and interactions used in the research; the collection of identifiable private information in the research; an underlying disease, disorder, or condition of the subject; and/or other circumstances unrelated to the research or any underlying disease, disorder, or condition of the subject.

8.2.2 Suspected Adverse Reactions and Adverse Reactions

A **suspected adverse reaction** is any adverse event for which there is a reasonable possibility that the drug caused the adverse event. Suspected adverse reactions are the subset of all adverse events for which there is a reasonable possibility that the drug caused the event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event.

"Reasonable possibility" may encompass any of the following scenarios:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug
- An aggregate analysis of specific events observed in a clinical trial (such as known consequences of the underlying disease or condition under investigation or other events that commonly occur in the study population independent of drug therapy) that indicates those events occur more frequently in the drug treatment group than in a concurrent or historical control group

An **adverse reaction** is any adverse event caused by a drug. Adverse reactions are a subset of all suspected adverse reactions where there is reason to conclude that the drug caused

the event.

A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

8.2.3 Serious Adverse Events

A **serious adverse event** (SAE) is any adverse experience occurring at any dose that results in any of the following outcomes:

- Results in **death.**
- Is a **life -threatening** adverse experience. The term life threatening in the definition of serious refers to an adverse event in which the subject was at risk of death at the time of the event. It does not refer to an adverse event which hypothetically might have caused death if it were more severe.
- Requires **inpatient hospitalization or prolongation of existing hospitalization (unless planned per protocol)**. Any adverse event leading to hospitalization or prolongation of hospitalization will be considered as Serious, UNLESS at least one of the following expectations is met:
    - The admission results in a hospital stay of less than 24 hours OR
    - The admission is pre-planned (e.g., elective or scheduled surgery arranged prior to the start of the study) OR
    - The admission is not associated with an adverse event (e.g., social hospitalization for purposes of respite care.

  However, it should be noted that invasive treatment during any hospitalization may fulfill the criteria of "medically important" and as such may be reportable as a serious adverse event dependent on clinical judgment. In addition, where local regulatory authorities specifically require a more stringent definition, the local regulation takes precedent.
- Results in **persistent or significant disability/incapacity**. The definition of disability is a substantial disruption of a person's ability to conduct normal life's functions.
- **Is a** congenital anomaly/birth defect**.**
- Is an **important medical event**. Important medical events that may not result death, be life threatening, or require hospitalization may be considered a serious adverse experience when, based upon appropriate medical judgment, they may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood disease or disorders, or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. The development of a new cancer is always considered an important medical event.

8.2.4 Adverse Event Evaluation

The investigator or designee is responsible for ensuring that all adverse events (both serious and non-serious) observed by the clinical team or reported by the subject which occur after the subject has signed the informed consent are fully recorded in the subject's medical records. Source documentation must be available to support all adverse events.

A laboratory test abnormality considered clinically relevant (e.g., causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, result in a delay or dose modification of study treatment, or judged relevant by the investigator), should be reported as an adverse event.

The investigator or sub-investigator (treating physician if applicable) will provide the following for all adverse events (both serious and non-serious):

- Event term (as per CTCAE version 5.0)
- Description of the event
- Date of onset and resolution
- **Expectedness of the toxicity**
- **Grade of toxicity**
- **Attribution of relatedness to the investigational agent (this must be assigned by an investigator, sub-investigator, or treating physician)**
- Action taken as a result of the event, including but not limited to; no changes, dose interrupted, reduced, discontinued, etc. or action taken with regard to the event, i.e. no action, received concomitant medication or other intervention, etc.
- Outcome of event

Descriptions and **grading scales** found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version **5.0** will be utilized for AE reporting, with the exception of grading provided in Section 6.4 for cytokine release syndrome.

An expected adverse event is an event previously known or anticipated to result from participation in the research study or any underlying disease, disorder, or condition of the subject. The event is usually listed in the Investigator Brochure, consent form or research protocol.

Adverse events determined to be due to the underlying disease progression will be recorded but will not be subject to the expedited reporting requirements outlined in Section 8.4. All AEs related to the disease and unrelated to the cell therapy administration will be reported annually to the FDA and IRB.

An unexpected adverse event is an adverse event not previously known or anticipated to result from the research study or any underlying disease, disorder, or condition of the subject. An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has

been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended.

Attribution is the relationship between an adverse event or serious adverse event and the study drug. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study drug.
- Probable – The AE is likely related to the study drug.
- Possible – The AE may be related to the study drug.
- Unlikely – The AE is doubtfully related to the study drug.
- Unrelated – The AE is clearly NOT related to the study drug.

8.3 Reporting Procedures for Serious Adverse Events

For the purposes of safety reporting, all adverse events will be reported that occur from the start of the infusion of the first dose of lymphodepletion regimen through 12 months after the infusion of CAR-T cells. Adverse events, both serious and non-serious, and deaths that occur during this period will be recorded in the source documents. All SAEs should be monitored until they are resolved or are clearly determined to be due to a subject's stable or chronic condition or intercurrent illness(es). Related AEs will be followed until resolution to baseline or grade 1 or stabilization.

8.4 Reporting Requirements

8.4.1 Adverse Event Reporting

In reporting adverse events, we will follow the regulations issued by the Food and Drug Administration addressing the safety reporting requirements for investigational new drug applications (INDs). Data from all subjects will be submitted to FDA to ensure a comprehensive and accurate review.

Should an AE occur, the patient will be monitored or followed until the AE is resolved. The AE description will include the description of the event, the specific symptoms of the event, the dates/duration of the symptoms, the grade, the outcome, and whether the AE was definitely, probably, or possibly related to the study or unrelated.

If a **serious AE** occurs, as defined in Section 8.2, the IRB will be notified, as per local policy. Unexpected AEs, defined as an AE that is not identified in nature, severity, or frequency in the study protocol; or the event was more serious than anticipated, will also be reported to the IRB, as per local policy. If a serious or unexpected AE occurs as a result of the treatment, the regimen will be evaluated for change/modifications and such changes will result in an IRB amendment. If an adverse event results in modification of the treatment, all active study participants will be informed and their willingness to continue in the study will be assessed.

Serious adverse events that are unexpected and associated with the use of the cell therapy product, but are not fatal or life-threatening, will be reported concurrently to the **FDA and IRB** as soon as possible, but not later than **15 calendar days** after the initial receipt of the information.

If, after further evaluation, an adverse event initially considered not to be associated with the use of the cell therapy product is subsequently determined to be associated, then the event will be reported concurrently to the **FDA and IRB** as soon as possible, but in no case later than **15 calendar** days after the determination is made.

Relevant additional clinical and laboratory data will become available following the initial serious adverse event report. Relevant follow-up information to an IND safety report will be submitted concurrently to the **FDA, IRB and the DSMB** as soon as the information is available and will be identified as such, i.e., "Follow-up IND Safety Report." If a serious adverse event occurs after the end of a clinical trial and is determined to be associated with the use of the cell therapy product, that event will be reported concurrently to the **FDA, IRB and the DSMB within 15 calendar** days of the determination.

Serious adverse events that are unexpected and associated with the use of the cell therapy product, and are fatal or life-threatening, will be reported concurrently to the **FDA and IRB** as soon as possible, but not later than **7 calendar days** after the initial receipt of the information. Relevant follow-up information to an IND safety report will be submitted concurrently to the **FDA, IRB and the DSMB** as soon as the information is available and will be identified as such, i.e., "Follow-up IND Safety Report."

8.4.2 Subsite Serious Adverse Event (SAE) Reporting Requirements

NOTE: External participating sites are not permitted to report directly to the OSU IRB or FDA (if applicable). NCH SAEs are to be reported to the OSU Principal Investigator and Multi-Center Trial Program (MCTP). The MCTP will facilitate submission of external site SAEs to the OSU IRB and FDA (if applicable).

All serious adverse events (SAEs) and other adverse events must be recorded on case report forms. In addition, all SAEs must be reported to the OSU Principal Investigator and MCTP within 24 hours of knowledge of the event using the FDA MedWatch 3500A mandatory reporting form. External participating sites must also submit the "SAE Submission Form" cover sheet (refer to the Supplemental Forms Document).

Copies of de-identified source documentation pertaining to the SAE must be submitted to OSU. If a patient is permanently withdrawn from the study because of a SAE, this information must be included in the initial or follow-up SAE report form.

All SAEs must be submitted to the local IRB per local IRB and institutional policy.

Upon request of additional data or information that is deemed necessary must be reported to OSU as soon as possible but no later than 5 calendar days.

9 PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 8.1.

9.1 Investigational Agents

9.1.1 Anti-CD19/20/22 Chimeric Antigen Receptor T (CAR-T) Cells

Solution preparation: The Anti-CD19/20/22 CAR-T cells are prepared at the James Hospital Cellular Therapy Laboratory (CTL). Once manufacturing is complete and release criteria are met, Anti-CD19/20/22 CAR-T cells can be released for immediate infusion or frozen for storage in cryomedia for infusion at a later date.

Storage requirements: When frozen, Anti-CD19/20/22 CAR-T cell products will be stored at the CTL in the vapor phase of liquid nitrogen.

Route of administration: Short intravenous infusion over 5 – 30 minutes.

Drug Procurement: The investigational agent will be manufactured at W.W. Williams Cellular Therapy Laboratory.

Packaging: The Anti-CD19/20/22 CAR-T cell product will be packaged in bags with an approximate content of 25-50 mL per bag, packed for transport in an outer protective package.

Labeling: Bags containing the Anti-CD19/20/22 CAR-T cell product will be labeled in accordance with applicable regulatory guidelines.

9.2 Commercial Agents

9.2.1 Cyclophosphamide

Chemical Name	Cyclophosphamide
Other Names	Cytoxan ®, Endoxan®, Neosar®, Procytox®, Revimmune®, Cycloblastin®
Classification	Alkylating agent
Molecular Formula	$C_7H_{15}Cl_2N_2O_2P$

Mode of action

The mechanism of action is thought to involve cross-linking of tumor cell DNA.

Metabolism

The liver is the major site of cyclophosphamide activation. Approximately 75% of the administered dose of cyclophosphamide is activated by hepatic microsomal cytochrome P450s including CYP2A6, 2B6, 3A4, 3A5, 2C9, 2C18 and 2C19, with 2B6 displaying the highest 4-hydroxylase activity. Cyclophosphamide is activated to form 4-hydroxycyclophosphamide, which is in equilibrium with its ring-open tautomer aldophosphamide. 4-hydroxycyclophosphamide and aldophosphamide can undergo oxidation by aldehyde dehydrogenases to form the inactive metabolites 4-ketocyclophosphamide and carboxyphosphamide, respectively. Aldophosphamide can undergo β-elimination to form active metabolite s phosphoramide mustard and acrolein. This spontaneous conversion can be catalyzed by albumin and other proteins. Less than 5% of cyclophosphamide may be directly detoxified by side chain oxidation, leading to the formation of inactive metabolites 2-dechloroethylcyclophosphamide. At high doses, the fraction of parent compound cleared by 4-hydroxylation is reduced resulting in non-linear elimination of cyclophosphamide in patients. Cyclophosphamide appears to induce its own metabolism. Auto-induction results in an increase in the total clearance, increased formation of 4-hydroxyl metabolites and shortened t1/2 values following repeated administration at 12- to 24-hour interval.

Product description

Cyclophosphamide for Injection, USP (lyophilized powder) is a sterile white cake containing cyclophosphamide and mannitol and is supplied in vials for single dose use.

Solution preparation

Reconstitution of Cyclophosphamide: Reconstitute Cyclophosphamide using 0.9% Sodium Chloride Injection, USP or Sterile Water for Injection, USP with the volume of diluent listed below in Table 9.2-1 Add the diluent to the vial and gently swirl to dissolve the drug completely.

Table 9.2-1: Reconstitution in preparation

Strength	Volume of Diluent	Cyclophosphamide Concentration
500mg	25 mL	
1g	50 mL	20mg per mL
2g	100 mL	

Dilution of Reconstituted Cyclophosphamide: Further dilute the reconstituted Cyclophosphamide solution to a minimum concentration of 2 mg per mL with any of the following diluents:

- 5% Dextrose Injection, USP
- 5% Dextrose and 0.9% Sodium Chloride Injection, USP 0.45% Sodium Chloride Injection, USP

To reduce the likelihood of adverse reactions that appear to be administration rate-dependent (e.g., facial swelling, headache, nasal congestion, scalp burning), cyclophosphamide should be injected or infused very slowly. Duration of the infusion also should be appropriate for the volume and type of carrier fluid to be infused.

Stability Reconstituted lyophilized cyclophosphamide is chemically and physically stable for 24 hours at room temperature or for six days in the refrigerator; it does not contain any antimicrobial preservative and thus care must to ensure the sterility of prepared solutions.

Route of administration Intravenous

Drug procurement Cyclophosphamide must be obtained from commercial sources.

9.2.2 Fludarabine

Chemical Name: Fludarabine phosphate

Other Names: Fludara®

Classification: Purine analog, antimetabolite

Molecular Formula: $C_{10}H_{13}FN_5O_7P$

Mode of Action: Fludarabine phosphate is rapidly dephosphorylated to 2-fluoro-ara-A and then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate, 2-fluoro-ara-ATP. This metabolite appears to act by inhibiting DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. The mechanism of action of this antimetabolite is not completely characterized and may be multi-faceted.

Metabolism: Phase 1 studies in humans have demonstrated that fludarabine

phosphate is rapidly converted to the active metabolite, 2-fluoro-ara-A, within minutes after IV infusion. Consequently, clinical pharmacology studies have focused on 2-fluoro-ara-A pharmacokinetics. After the five daily doses of 25 mg 2-fluoro-ara-AMP/m2 to cancer patients infused over 30 minutes, 2-fluoro-ara-A concentrations show a moderate accumulation. During a 5-day treatment schedule, 2-fluoro-ara-A plasma trough levels increased by a factor of about 2. The terminal half-life of 2-fluoro-ara-A was estimated as approximately 20 hours. In vitro, plasma protein binding of fludarabine ranged between 19% and 29%.

Product description: Fludara for injection is supplied as a white, lyophilized solid cake. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2.

Fludara for injection is supplied in a clear glass single dose vial (6 ml capacity).

Solution preparation: Fludarabine phosphate for injection should be prepared for parenteral use by aseptically adding sterile water for injection. When reconstituted with 2 ml of sterile water for injection, the solid cake should fully dissolve in 15 seconds or less; each ml of the resulting solution will contain 25 mg of fludarabine phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7.7. The pH range for the final product is 7.2-8.2. In clinical studies, the product has been diluted in 100 or 125 cc of 5% dextrose injection or 0.9% sodium chloride.
Reconstituted fludarabine phosphate for injection contains no antimicrobial preservative and thus should be used within 8 hours of reconstitution. Care must be taken to assure the sterility of prepared solutions. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

Storage requirements*:* Store under refrigeration, between 2-8°C (36-46°F).

Stability: When stored as directed, the powder for injection is stable for at least 18 months after the date of manufacture. While early stability studies reported that the powder for injection was stable for at least 36 months when stored at 22–25°C, more recent studies employing assays with increased sensitivity have shown that the drug is less stable than this; therefore, the manufacturer currently recommends that fludarabine phosphate powder for injection not be stored at room temperature.

Fludarabine phosphate is relatively stable in aqueous solutions, with optimal stability occurring at an approximately neutral pH. When reconstituted to a final concentration of 25 mg/mL, aqueous solutions of the drug are stable for at least 16 days at room temperature and normal light conditions. When diluted to a final concentration of 1 mg/mL, the drug is compatible in these diluents for at least 16 days at room temperature and normal light conditions. However, because such reconstituted and diluted fludarabine phosphate solutions contain no preservatives, the manufacturer recommends that they be used within 8 hours after preparation.

Route of administration*:* Intravenous

Drug Procurement: Fludarabine must be obtained from commercial sources.

Fludarabine must be obtained from commercial sources and is available in 50 mg/6mL capacity vials. **The cost of this agent will be the subject's responsibility.**

10 EXPLORATORY or CORRELATIVE STUDIES

We will investigate the changes in cytokine serum concentrations, Anti-CD19/20/22 CAR-T cell subpopulations and their correlation with adverse events, response and Anti-CD19/20/22 CAR-T cell persistence (measured by flow cytometry and qPCR). We will also investigate the correlation of CD19, CD20 and/or CD22 expression on baseline tissue or peripheral blood with disease response and incidence of adverse events. Lastly, we will also characterize the extracellular vesicles extracted from transduced and non-transduced T cells. **Please see Lab manual with a Correlative Calendar for specifics regarding timing of sample collection. Changes will be updated in correlative calendar.**

10.1 Methods

10.1.1 Cytokine plasma concentrations

Patient blood samples (10ml peripheral blood) will be collected at timepoints per study calendar and corelative sample calendar. If CRS/ICANS occurs at any of these timepoints, samples need not be duplicated.

Samples will be collected and processed by the SOPs in Dr. Alinari's lab. Sample processing will include ficoll separation to isolate plasma and mononuclear cells. The plasma will be frozen -80ºC for subsequent analysis using Meso Scale Discovery panels, with cytokines measured including Interleukin (IL)-1 Ra, IL-2 Rα, IL-6, IL-8, IL-10, IL-15, Granzyme A and B, Interferon gamma (IFNγ), chemokine (C-C motif) ligand 2 (CCL2), IFNγ – induced protein 10 (IP-10).

Contact information for the processing of blood samples for cytokine concentrations:

Lapo Alinari MD, PhD

Ohio State University Comprehensive Cancer Center
Rm 481D, Wiseman Hall
410 W 12th Ave
Columbus OH, 43210

10.1.2 Flow cytometry-based phenotyping and persistence of Anti-CD19/20/22 CAR-T cells

We will use flow cytometry to characterize the Anti-CD19/20/22 CAR-T cell product prior to infusion as well as to characterize the changes of Anti-CD19/20/22 CAR-T cell phenotype after infusion and *in vivo* expansion. Samples will include: A) Anti-CD19/20/22 CAR-T cell product and B) Patient samples (10ml peripheral blood): at timepoints per study calendar and corelative sample calendar.

In addition, flow cytometry will be used to measure the persistence of CAR-T cells at the time points listed above. Flow cytometry assays will be done at the time points delineated above, but the assay will not be performed after if it is not detectable in two consecutive time points.

We will perform flow cytometry testing of the Anti-CD19/20/22 CAR-T cells to assess the composition of the product, the transduction efficiency and viability, the differentiation status, proliferative capacity, immune cell sub population , exhaustion and activation status. The flow panels to be used for basic characterization are listed in Table 10.1.

Table 10.1: Flow cytometry-based phenotyping and persistence of Anti-CD19/20/22 CAR-T cells

Dye	Cellular Composition and Viability	Transduction and Viability	Differentiation	Proliferative Capacity	Exhaustion	Activation
VioBlue	CD45	CD45	CD62L	CD27	CD223	CD154
VioGreen	CD4	CD4	CD4	CD4	CD4	CD4
FITC	CD3	CD3	CD3	CD3	CD3	CD3
PE	CD16/CD56	CAR*	CAR*	CAR*	CAR*	CAR*
PerCP	7-AAD	7-AAD	7-AAD	7-AAD	7-AAD	7-AAD
PE-Vio770	CD20		CD45RO	CD279	CD279	CD25**
APC	CD14	CD14	CD95	CD127	CD366	CD137
APC	CD8	CD8	CD8	CD8	CD8	CD8

Flow cytometry will be performed in the Lab of Dr. Alinari.

10.1.3 Anti-CD19/20/22 CAR-T Cell Persistence Assays

We will apply the methods described by Till and colleagues[59,60] and Kochenderfer and colleagues for human anti-CD19 CAR-T cell quantification using flow quantitative PCR.[61] Samples will be collected at timepoints per study calendar and corelative sample calendar. NOTE: Vector persistence assays will be done at the time points delineated above until no longer detectable in 2 consecutive time points.

For each patient sample collected, peripheral blood mononuclear cells (PBMCs) will be

isolated using Ficoll centrifugation and cryopreserved. DNA will be extracted from thawed sample and amplified in duplicate with a primer and probe set specific for the Anti-CD19/20/22 CAR. Real time PCR will then be conducted using serial dilutions of DNA from infused T cells from each patient into pre-treatment DNA from the same subject. A standard curve will be done performing qPCR on this DNA. The percentage of infused T cells expressing the Anti-CD19/20/22 CAR will be measured by flow cytometry using anti-Fab antibody staining. The standard curve will be constructed in units of % CAR+ T cells, with the highest point (100%) corresponding to that of undiluted infused T-cell DNA. The percentage of PBMC that contain CAR gene at each time point will be determined by comparing the qPCR results obtained from each time point with those of the standard curve specific to each patient. After the percentage of CAR+ PBMC is determined by PCR, the absolute number of CAR+ PBMC will be calculated by multiplying the percentage of CAR+ PBMC (extracted from the standard curve) by the sum of the absolute number of blood lymphocytes and monocytes.
NOTE: Method and reagent modifications to this process may be required though the course of the trial in order to improve the performance of the assay.

These analyses will be conducted at the Lab of Dr. Alinari, who will work with other OSU cores or laboratories as needed to accomplish the research goals. Two laboratories outside OSU will receive samples, as follows:

Lentigen/ Miltenyi Biotec
1201 Clopper Road
Gaithersburg, MD, 20878

Sample Types to be sent to Miltenyi (subject to availability):

Apheresis material pre-manufacturing, in process samples from Prodigy CAR T manufacturing, final CAR T product pre-infusion.

Samples after CAR T infusion : i.e. peripheral blood samples form patients from day 28 post-infusion, and further follow up days. Biopsy samples if available.

Intended use: Miltenyi will use samples for research only (unidentified samples). Samples may be used for immunohistochemistry, immunofluorescent imaging, including ultra-high-content imaging, qPCR analysis, real time qPCR analysis, single cell sequencing and bulk sequencing, flow cytometry, cell based assays.

Cryogenic vials (Corning - 430487 - Corning 1.2 mL Internal Threaded Polypropylene Cryogenic Vial, Self-Standing with Conical Bottom, Item #: 10387282MFR SKU: 430487 or comparable). Desired cell number per vial: 1 million to 10 million (maximum of 2E7 cells/ml), in 1 ml. Desired amount: 1-3 vials of 1-10 million cells/vial for each sample, or a smaller cell number per vial, whichever is available.

Rafick Sekaly, PhD
Attn: Linda Roback
Emory University
1462 Clifton Rd.
Dental Bldg.
Room 431
Atlanta, GA 30322
phone: 404-788-1468

Dr. Sekaly's lab will investigate the role of T_{SCM} cells have in manufacturing, CAR T persistence and clinical outcomes. Dr. Sekaly is internationally recognized as an expert in the field of T cell biology.

Unidentified samples to be sent: 5 plasma tubes (500uL each) and ~2-4 vials of cryopreserved PBMCs (10million cells vial) - isolated from whole blood collected in ACD tubes.

10.1.4 CD19, CD20 and CD22 expression studies

Baseline tissue samples and peripheral blood are evaluated by immunohistochemistry and/or flow cytometry for CD19, CD20 and CD22 expression. Only subjects diagnosed as CD19, and/or CD20, and/or CD22 positive will be enrolled in this trial. The percentages of CD19/20/22 positive malignant cells measured on pre-enrollment tissue and/or flow cytometry will be recorded to determine whether there is correlation with disease response to Anti-CD19/20/22 CAR-T cells as well as with the incidence of adverse events, including but not limited to CRS and ICANS. Comparisons of CD19/20/22 expression levels will be done between responders and non – responders using non parametric tests, including McNemar's test when data is considered binary, exact Fisher's test for proportion comparison and Mann-Whitney's test to compare means between groups.

10.1.5 Extracellular Vesicles (EVs) studies

Extracellular Vesicles (EVs) derived from 1) non-transduced T cells and 2) CD19/20/22 CAR-T cells. EVs are extracted from samples via ultra-centrifugation. Phenotypic analyses, including flow cytometry, Western blotting, quantitative reverse transcriptase polymerase chain reaction (qRT-PCR), and nanoparticle tracking analysis (NTA), will be performed on extracted EVs to determine 1) the efficacy of the extraction process, and 2) the size and characteristics of the EVs, including differences in EV 'cargo' between samples. The EVs will be shipped frozen to Dr. Renato Cunha and Amanda Corveloni, collaborators in Brazil. Results of these analyses are for research information use only and will not be used for diagnostic or treatment purposes.

Samples will be sent to:
ATTN: Dr. Renato Cunha & Amanda Corveloni
Faculdade de Medicina de Ribeirao Preto
USP. Av Bandeirantes, 3900. Anexo A
Bloco B, Primeiro Andar. Sala 40. CEP
14049-900. Ribeirão Preto-SP, Brazil

11 STUDY PARAMETERS AND CALENDAR

11.1 Study Parameters

11.1.1 Screening Evaluation

Screening studies and evaluations will be used to determine the eligibility of each subject for study inclusion. All evaluations (with the exception of echocardiogram and tissue biopsy or peripheral blood for (CD19/20/22) targeted antigen expression by flow) must be completed ≤ 28 days prior to patient enrollment on protocol.

11.1.2 Treatment Period

As this is a cellular therapy clinical trial, the treatment period will include the lymphodepletion regimen (starting on day -6), followed by the CAR-T cell infusion (day 0), with subsequent follow up, including the initial safety monitoring period (Day 0 to Day 30) and the survival observation period (day 30 and beyond). The trial observation period for dose limiting toxicities will conclude at day 30, while the long-term post-infusion follow-up will conclude 12 months after the CAR-T cell infusion. The study windows for baseline through day 30 visits are ± 3 days, for laboratory tests and imaging studies. Correlative labs after day 14 have a window of ±3 days; prior to day 14, all efforts should be made to collect samples on study visit day. All study windows for visits, laboratory studies, tests and correlatives after day 30 to 12 months/end of study are ± 14 days.

Day - 6

- Cyclophosphamide treatment (Cohorts A and B), Cyclophosphamide and Fludarabine (Cohort C)
- Vital signs (body temperature, respiratory rate, blood pressure, and heart rate)
- Physical examination
- Performance Status (PS)
- Assessment of adverse events
- Clinical laboratory assessment:
    - CBC with differential *(includes white blood cell count, hemoglobin, hematocrit, and platelets)*
    - CMP *(includes sodium, potassium, chloride, calcium, bicarbonate, blood urea nitrogen, creatinine, glucose, total bilirubin, alkaline phosphatase, ALT, AST, albumin, total protein, LDH)*
    - PT/PTT as clinically indicated
    - magnesium
        - ferritin
- Serum pregnancy test for female patients of childbearing potential. Must be resulted prior to initiating treatment.
- Peripheral blood correlative sample collection (as defined in the Lab manual)

- NOTE: In cases when CAR-T cell manufacturing has issues, exact timing of cell infusion may be delayed up to a week.

Days -5 to Day -4

- Fludarabine treatment (All Cohorts), Cyclophosphamide treatment on day -5 (Cohort C)
- Vital signs
- PS
- Assessment of adverse events
- NOTE: In cases when CAR-T cell manufacturing requires extension for up to 96 hours, exact timing of lymphodepletive regimen may correspond up to 96 hours earlier.

Day – 3

- Fludarabine treatment
- Vital signs
- PS
- Clinical laboratory assessment:
    - CBC with differential
    - CMP
    - PT/PTT as clinically indicated
    - magnesium
    - ferritin
- NOTE: In cases when CAR-T cell manufacturing requires extension for up to 96 hours, exact timing of lymphodepletive regimen may correspond up to 96 hours earlier.

Day -2

- Correlative blood collection per sample collection calendar (Appendix B)

Day – 1 or Day 0

- Pre-infusion eligibility re-check (see Section 4)

Day 0

- Vital signs
- Physical examination
- PS
- Neurologic examinationICANS and CRS assessment
- Assessment of adverse events
- Clinical laboratory assessment (to be drawn prior to CAR-T cell infusion):
    - CBC with differential
    - CMP
    - PT/PTT
    - magnesium
    - ferritin

- CAR-T Cell infusion
- Peripheral blood correlative sample collection
- Monitoring of CRS will be done as per Institutional standard operating procedures

  NOTE: In cases when CAR-T cell manufacturing has issues, exact timing of cell infusion may be delayed up to a week.

Day +1 to Day 6

- Vital signs
- PS
- Neurologic examination (day 6 only)
- ICANS and CRS assessment
- Assessment of adverse events
- Clinical laboratory assessment:
  - CBC with differential
  - CMP
  - PT/PTT as clinically indicated
  - magnesium
  - ferritin
- Peripheral blood correlative sample collection

Day +7 (Cohort B and Cohort C only)

- Vital signs
- Physical examination
- PS
- ICANS and CRS assessment
- Neurologic examination
- Assessment of adverse events
- Clinical laboratory assessment;
  - CBC with differential
  - CMP
  - PT/PTT as clinically indicated
  - magnesium
  - ferritin
- Optional bone marrow biopsy, as clinically indicated (including aspirate and 2 tubes for research) (may be done on day +6 or +7)
- Pre-infusion eligibility re-check (see Section 4)
- CAR-T Cell infusion #2 (Cohort B and Cohort C)
- Correlative assay sample collection (before CAR-T cell infusion)
- NOTE: if conditions for day 7 infusion are not met, infusion can be delayed until day 10 providing all criteria are met.

Day +9 (Cohort B and Cohort C only)

- Correlative assay sample collection (Cohort B and Cohort C)
- NOTE: in the event that infusion #2 is delayed, the time to draw correlative samples will also be delayed. Correlative sample are to be drawn 2 days after the infusion.

Day +14, Day +21, Day +30 (± 3 day window)

- Vital signs
- Physical examination
- PS
- Neurologic examination
- Assessment of adverse events
- Clinical laboratory assessment:
    - CBC with differential
    - CMP
    - PT/PTT as clinically indicated
    - magnesium
    - ferritin
- Response assessment (Day 30 only), as clinically indicated
    - PET/CT for FDG avid lymphomas; CT scan (chest, abdomen and pelvis, neck if previously involved) for CLL and non- FDG avid lymphomas. No imaging studies required for ALL patients unless lymphoblastic lymphoma.
    - Bone marrow biopsy with flow cytometry and molecular studies for ALL and CLL, as clinically indicated. Two (2) additional tubes of bone marrow aspirate will be collected.
    - Note: given supply chain issues and unpredictable availability of specific tubes, we may use EDTA or Na Hep based on availability.
    - Peripheral blood flow cytometry.
    - Bone marrow biopsy for lymphoma (if previously involved by disease) based upon disease-specific studies described in Section 12. Two (2) additional tubes of bone marrow aspirate will be collected.
- Peripheral blood correlative sample collection

Day + 60 (Follow up visits have a ± 14 day window)

- Vital signs
- Physical examination
- PS
- Assessment of adverse events
- Clinical laboratory assessment
    - CBC with differential
    - CMP
    - PT/PTT as clinically indicated
    - magnesium
    - ferritin
- Response assessment
    - PET/CT (as clinically indicated) for FDG avid lymphomas (may perform CT scan if subject already achieved remission and no suspicion or recurrence, with prior discussion with principal investigator); CT scan (chest, abdomen and pelvis, neck if previously involved) for CLL and non- FD G avid lymphomas. No imaging studies required for ALL patients unless

lymphoblastic lymphoma.
- Bone marrow biopsy with flow cytometry and molecular studies for ALL, as clinically indicated. Two (2) additional tubes of bone marrow aspirate will be collected.
- Bone marrow biopsy for lymphoma (if previously involved by disease and not in complete remission in prior bone marrow biopsy), as clinically indicated based upon disease- specific studies described in Section 12. Two (2) additional tubes of bone marrow aspirate will be collected.
- Bone marrow biopsy for CLL if not in complete remission in prior bone marrow biopsy, as clinically indicated. Two (2) additional tubes of bone marrow aspirate will be collected.
- Note: given supply chain issues and unpredictable availability of specific tubes, we may use EDTA or Na Hep based on availability.
- Peripheral blood flow cytometry.

- Peripheral blood correlative sample collection

Day +90 (Follow up visits have a ± 14 day window)

- Vital signs
- Physical examination
- PS
- Assessment of adverse events
- Record concomitant medications
- Clinical laboratory assessment
  - CBC with differential
  - CMP
  - PT/PTT as clinically indicated
  - magnesium
  - ferritin
- Response assessment
  - PET/CT (as clinically indicated) for FDG avid lymphomas (may perform CT scan if subject already achieved remission and no suspicion or recurrence, with prior discussion with principal investigator); CT scan (chest, abdomen and pelvis, neck if previously involved) for CLL and non- FD G avid lymphomas. No imaging studies required for ALL patients unless lymphoblastic lymphoma.
  - Bone marrow biopsy with flow cytometry and molecular studies for ALL as per standard of care. Two (2) additional tubes of bone marrow aspirate will be collected
  - Bone marrow biopsy for lymphoma (if previously involved by disease and not in complete remission in prior bone marrow biopsy), as clinically indicated based upon disease- specific studies described in Section 12. Two (2) additional tubes of bone marrow aspirate will be collected.
  - Bone marrow biopsy for CLL if not in complete remission in prior bone marrow biopsy. Two (2) additional tubes of bone marrow aspirate will be collected.

  - Note: given supply chain issues and unpredictable availability of specific tubes, we may use EDTA or Na Hep based on availability.
  - Peripheral blood flow cytometry.

- Peripheral blood correlative sample collection

Day +6 months, Day +12 months* (Follow up visits have a ± 14 day window)

- Response assessment.
- Vital signs
- PS
- Physical Exam
- Assessment of adverse events
- Record concomitant medications
- Clinical laboratory assessment
  - CBC with differential
  - CMP
  - PT/PTT as clinically indicated
  - magnesium
  - ferritin
- Response assessment
  - PET/CT (as clinically indicated) for FDG avid lymphomas (may perform CT scan if subject already achieved remission and no suspicion or recurrence, with prior discussion with principal investigator); CT scan (chest, abdomen and pelvis, neck if previously involved) for CLL and non- FD G avid lymphomas. No imaging studies required for ALL patients unless lymphoblastic lymphoma.
  - Bone marrow biopsy with flow cytometry and molecular studies for ALL as per standard of care. Two (2) additional tubes of bone marrow aspirate will be collected.
  - Bone marrow biopsy for lymphoma (if previously involved by disease and not in complete remission in prior bone marrow biopsy), as clinically indicated based upon disease- specific studies described in Section 12. Two (2) additional tubes of bone marrow aspirate will be collected.
  - Bone marrow biopsy for CLL if not in complete remission in prior bone marrow biopsy, as clinically indicated. Two (2) additional tubes of bone marrow aspirate will be collected. Peripheral blood flow cytometry.
  - Given supply chain issues and unpredictable availability of specific tubes, we may use EDTA or Na Hep based on availability.

- Peripheral blood correlative sample collection
- Long-term follow up visit checklist (Appendix 5) (only for 12-month visit).

NOTE: (as suggested by the FDA)
Even though all components in the vector used for Trispecific CART except

for ScFv 19 and ScFv 20 are human, and human components are not supposed to induce immunogenicity, we propose to test a subset of patients for immunogenicity in batched samples at day + 90 and day + 180 using a commercial assay (such as the ones described here (https://www.precisionformedicine.com/specialty-lab-services/bioanalytical-testing/immunogenicity-testing/). We would test in a limited number of patients due to budgetary constraints

NOTE: ADDITIONAL SAMPLES FOR CORRELATIVE ASSAYS (CRS or ICANS)

- In the event a subject develops an immune mediated adverse event, including CRS and/or ICANS, additional samples for correlative assays (CAR-T cell detection, cytokines, T cell subpopulations) will be drawn. The samples will be drawn at the onset and follow up of the immune adverse event.
- In subjects who develop CRS or ICANS, a blood sample collected on a 5 mL EDTA tube will be collected for plasma isolation for future identification of potential viral etiologic agents (Correlative study calendar in lab Manual). Baseline samples are requested on the study calendar.

*Or End of Study Visit: If patients are removed from the study prior to 12 months

Long Term Follow Up (24, 36, 48, 60 months) (LTFU visits have a ± 30 day window)

- Peripheral blood correlative sample (vector/provirus persistence assay) collection. Annually after month 12.
- Long term follow up visit checklist (Appendix 5)

Long Term Follow Up (Years 6 – 15 after CAR-T infusion) (LTFU visits have a ± 30 day window)

- Peripheral blood correlative sample (vector/provirus persistence assay) collection. Annually.
- Long term follow up visit checklist (Appendix 6)

Safety Follow Up

If patient has disease progression or relapse, correlative samples and adverse events will continue to be collected as outlined in study calendar.

11.2 Calendar

	Baseline ≤ 28 days	Day -7 (upto day - 12) for fresh; Day – 30 to day -7 for cryopreserved	Day - 6	Day -5	Day -4	Day -3	Day 0 or -1	Day 0	Day 1 – 6	Day 7	Day 14 ± 3d	Day 21 ± 3d	Day 30 ± 3d	Day 60 ± 14d	Day 90 ± 14d	6 months ± 14d	12 months ± 14d	LTFU[10] Years 2–5 years ± 30 days	LTFU[10] Years 6– 15 years ± 30 days
Tissue biopsy (Lymph nodes) or peripheral blood for CD19/20/22 status by Flow cytometry or Immunohistochemical staining, as standard of care, if feasible. Results of last available test are allowed outside screening window.	X																		
Peripheral blood for CD19 CAR-T circulating levels by Flow cytometry[17]	X																		
Autologous peripheral blood cell collection[1]		X																	
Cyclophosphamide[2]			X	X[18]															
Fludarabine[2]			X[18]	X	X	X													
CAR-T cell infusion								X		X[12]									
Informed Consent	X																		
Inclusion/Exclusion	X																		
Pre-infusion Inclusion Criteria							X			X[12]									
Medical History	X												X	X	X	X	X		
Disease status/Response assessment[3]	X												X	X	X	X	X		
CBC w/diff	X		X			X		X	X	X	X	X	X	X	X	X	X		
Serum Chemistries[4] [4]	X		X			X		X	X	X	X	X	X	X	X	X	X		
Ferritin	X		X			X		X	X	X	X	X	X	X	X	X	X		
HIV	X																		
βHCG[5]	X		X																

Imaging studies[6,7]	X												X	X	X	X	X		
Performance Status	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X		
Vital Signs	X		X	X	X	X		X	X	X	X	X	X	X	X	X	X		
Physical Exam	X		X					X	X	X	X	X	X	X	X	X	X		
Neurologic examination								X[8]	X[9]	X	X	X							
ICANS and CRS assessment								X	X	X	X[19]	X	X						
Concomitant Meds	X															XX			
AE query		XX																	
LVEF (measured with Echocardiogram or MUGA)[11]	X																		
Bone Marrow Biopsy and Aspirate[13]	X									X[16]			X	X	X	X	X		
Hepatitis B and C panel[14]	X																		
Long term follow-up visit checklist																	X	X	
Long term follow-up query checklist																			X

Correlative studies including schedule are included in the lab manual and correlative calendar.

1. Autologous mononuclear cell collection to be done per institutional standards of Arthur G. James Cancer Hospital. **PLEASE NOTE ADDITIONAL REQUIREMENT** to collect CBC and CD3 % (only if indicated) within 1 week of cell collection. Autologous collection will occur anytime between day -12 to day -7 for fresh product infusions (variations in time will depend on available quantity of CAR-T cells after manufacturing period). If cryopreserved infusion is planned due to clinical indication or scheduling constraints, autologous collection can occur anytime between day -30 and day -7.
2. Cyclophosphamide is given on day -6. If extension of CAR-T manufacturing is required to achieve planned dose, time points of conditioning regimen may vary in relation to day 0 up to 96 hours (i.e. extension of culture from 6 days to 12 days). To be based on disease specific studies listed below and per Section 12.
3. PET/CT scans for FDG avid lymphomas, CT scans for non-FDG avid lymphomas; imaging studies are considered standard of care
4. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, lactate dehydrogenase, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium**,** Mg, , PT, and PTT.
5. Only for female participants of childbearing potential.
6. For follow up imaging studies: FDG PET with separate CTs for diagnostic measurements if previous PET/CT scan was positive; CT scan (chest, abdomen and pelvis, neck if previously involved) if previous PET/CT scan was negative and for non-FDG avid lymphomas. PET/CTs will be done as clinically indicated.
7. See calendar of Correlative Sample Collection Calendar in lab Manual and Correlative and Monitoring Assay Calendar.
8. To be done After infusion on Day 0 and prior to infusion on Day 7 for the Cohort B and Cohort C (infusion #2)
9. To be done on day 6
10. Long-term follow up. See Appendix 5 and Appendix 6 for LTF visit and query checklists, respectively.
11. LVEF may be greater than 28 days if no therapy has occurred since imaging
12. 2nd CAR T-Cell infusion administered for Cohort B and Cohort C only. NOTE: if conditions for day 7 infusion are not met, infusion can be delayed until day 10 providing all criteria are met.
13. All marrow samples are per standard of care for patient disease in either cohort at discretion of treating physician. In addition, standard of care Clonoseq MRD assays at the study specified time points Day 30, 90, 6 and 12 months as clinically indicated for DLBCL and Acute lymphoblastic leukemia patients (Cohort B and Cohort C).
14. Includes serologic testing for baseline viral etiologic agents (Hep B PCR and hep C PCR).
15. Provirus persistence samples to be collected annually until vector sequences no longer detectable by qPCR
16. Day 7 Bone Marrow Biopsy is optional
17. Patients who received prior CAR-T cells only. Test may be performed at OSU for patients who received commercial CAR-T cells. Test results from other institutions are acceptable for patients who received non-commercial CAR-T.
18. Pediatric patients in Cohort C only
19. For Cohorts B and C: ICANs and CRS assessments to be completed daily through Day 14 while patients are admitted

12 MEASUREMENT OF EFFECT

12.1 Lymphoma Response Criteria

The 2014 Lugano Response for Malignant Lymphoma will be used the following categories of response:

NOTE: These criteria are based upon the criteria from the Revised Response Criteria for Malignant Lymphoma.[62]

The criteria use the following categories of response: Complete Response (CR), Partial Response (PR), Stable Disease (SD), Relapse and Progression (PD). In the case of stable disease, follow-up assessments must have met the SD criteria at least once after entry to that step at a minimum interval of eight weeks.

The following guidelines are to be used for establishing tumor measurements at the baseline of each treatment step and for subsequent comparison:

- The six largest measurable nodes or extranodal masses must be identified as **Target Lesions** at baseline**.**
- If there are 6 or fewer measurable nodes and extranodal masses, all must be listed as **Target Lesions**
- If there are more than 6 involved measurable nodes or extranodal masses, the 6 largest nodes or extranodal masses should be selected as **Target Lesions** according to the following features: a) they should be clearly measurable in at least two perpendicular measurements; b) they should be from as disparate regions of the body as possible; and c) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved. When there are more than 6 involved measurable nodes or extranodal masses, any lesions that are not included within these 6 Target Lesions will be considered non- measured lesions.
- **Nonmeasured lesions:** Any disease not selected as measured, dominant disease and truly assessable disease should be considered not measured. These sites include any nodes, nodal masses, and extranodal sites not selected as dominant or measurable or that do not meet the requirements for measurability but are still considered abnormal, as well as any site of suspected disease that would be difficult to follow quantitatively with measurement, including pleural effusions, ascites, bone lesions, leptomeningeal disease, abdominal masses, and other lesions that cannot be confirmed, measured or followed by imaging.
- Measurements for all Target Lesions will be reported at baseline of each treatment step. Measurements for non-measured lesions are not required.
- The lymph nodes or extranodal masses selected as **Target Lesions** for measurement should be measured in **two perpendicular diameters**, one of which is the longest perpendicular diameter. The lymph nodes should be measured in centimeters to the nearest one tenth of a centimeter (e.g. 2.0 cm, 2.1cm, 2.2 cm, etc.). **A measurable node must have a longest diameter (LDi) greater than 1.5 cm**. Measurable extranodal disease (e.g., hepatic nodules) may be included in

the six representative, measured lesions. A measurable extranodal lesion should have an LDi greater than 1.0 cm.

· The two measured diameters of each Target Lesion should be multiplied giving a product for each nodal site or extranodal mass. The product of each site should be added, yielding the sum of products of the diameters (SPD). The SPD will be used in determining the definition of response for those who have less than a complete response.

· PET-based response should use the following 5-point scale: PET 5-point scale: 1, no uptake above background; 2, uptake ≤ mediastinum; 3, uptake > mediastinum but ≤ liver; 4, uptake moderately > liver; 5, uptake markedly higher than liver and/or new lesions; X, new areas of uptake unlikely to be related to lymphoma.

12.1.1 Complete Response

Complete disappearance of all detectable clinical evidence of disease, and disease-related symptoms if present prior to therapy.

- PET-CT Based Criteria
  - · Complete metabolic response with a 5– point scale score of 1, 2 or 3, with or without a residual mass.
  - · In patients with bone marrow involvement before treatment, there must be no residual FDG uptake in the marrow.
  - · In patients with a typically FDG-avid lymphoma with no pre- treatment PET scan, or for lymphomas for which the PET scan was positive prior to therapy: a post-treatment residual mass of any size is permitted as long as it is PET-negative.
- CT Based Criteria
  - · For variably FDG-avid lymphomas without a pretreatment PET scan, or if a pretreatment PET scan was negative: all lymph nodes and extranodal masses must have regressed on CT to normal size (≤ 1.5 cm in their greatest transverse diameter for nodes > 1.5 cm prior to therapy). Previously involved nodes that were 1.1-1.5 cm in their long axis and > 1.0 cm in their short axis prior to treatment must have decreased to ≤ 1 cm in their short axis after treatment.
  - · The spleen and/or liver, if considered enlarged prior to therapy on the basis of a physical examination or CT scan, should not be palpable on physical examination, and nodules related to lymphoma should disappear. However, no normal size can be specified because of the difficulties in accurately evaluating splenic and hepatic size and involvement. For instance, a spleen considered normal size may contain lymphoma, whereas an enlarged spleen may not necessarily reflect the presence of lymphoma, but variations in anatomy, blood volume, the use of hematopoietic growth factors, or other causes.
  - · If the bone marrow was involved by lymphoma prior to treatment, the infiltrate must have cleared on repeat bone marrow biopsy. The biopsy sample on which this determination is made must be adequate (with a goal of > 20 mm unilateral core). If the sample is indeterminate by morphology, it should be

negative by immunohistochemistry. A sample that is negative by immunohistochemistry but demonstrating a small population of clonal lymphocytes by flow cytometry will be considered a CR until data become available demonstrating a clear difference in patient outcome.

§ **NOTE:** Complete Remission/unconfirmed (CRu): Using the above definition for CR and that below for PR eliminates the category of CRu.

12.1.2 Partial Response (PR)

The designation of PR requires all of the following:

- PET-CT Based Criteria
  - · Partial metabolic response with reduced uptake compared with baseline AND a 5 point scale score of 4 or 5.
  - · For a typically FDG-avid lymphoma with no pretreatment PET scan or one that was PET-positive prior to therapy, the post-treatment PET should be positive at any previously involved sites.
  - · In patients with bone marrow involvement before treatment, Residual uptake higher than uptake in normal marrow but reduced compared with baseline (diffuse uptake compatible with reactive changes from chemotherapy allowed). If there are persistent focal changes in the marrow in the context of a nodal response, consideration should be given to further evaluation with MRI or biopsy or an interval scan.
- CT Based Criteria
  - · For variably FDG-avid lymphomas/FDG-avidity unknown, without a pretreatment PET scan, or if a pretreatment PET scan was negative, CT scan criteria should be used.
  - · A ≥ 50% decrease in sum of the product of the diameters (SPD) of up to 6 of the largest Target Lesions. These nodes or masses should be selected according to the following: (a) they should be clearly measurable in at least 2 perpendicular dimensions; if possible, they should be from disparate regions of the body; (b) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.
  - · No increase in the size of other nodes, liver or spleen.
  - · Bone marrow assessment is irrelevant for determination of a PR if the sample was positive prior to treatment. However, if positive, the cell type should be specified, e.g., large-cell lymphoma or small cleaved cell lymphoma.
  - · No new sites of disease.
  - · Patients who achieve a CR by the above criteria, but who have persistent morphologic bone marrow involvement will be considered partial responders.
  - · When the bone marrow was involved before therapy and a clinical CR was achieved, but with no bone marrow assessment after treatment, patients should be considered partial responders.

12.1.3 Stable Disease (SD)

- PET-CT Based Criteria:
  - Absence of metabolic response, with a score of 4 or 5 AND no significant change from baseline at interim or end of treatment.
  - In patients with bone marrow involvement before treatment, there must be no change from pre-treatment PET scan.
  - No new areas of FDG uptake.
- CT Based Criteria
  - For variably FDG-avid lymphomas/FDG-avidity unknown: For patients without a pretreatment PET scan or if the pre-treatment PET was negative, there must be no change in the size of the previous lesions on the post-treatment CT scan.
  - Less than 50% decrease from baseline in SPD of up to 6 Target Lesions.
  - No increase in organ enlargement and non-measurable lesions compatible with progressive disease.

12.1.4 Progression (PD) and Relapse

- PET-CT Based Criteria
  - Progressive metabolic disease:
  - Individual target nodes and nodal masses must present increase intensity of uptake from baseline, with a 5 point score of 4 or 5, or
  - Extranodal lesions with new FDG-avid foci consistent with lymphoma at interim or end of treatment assessment, or
  - New FDG-avid foci consistent with lymphoma rather than another etiology (e.g. Infection, inflammation). If uncertain regarding the etiology of new lesions, a biopsy or repeat imaging scan should be considered.
- CT Based Criteria
  - For determination of relapsed and progressive disease, lymph nodes should be considered abnormal if the long axis is more than 1.5 cm, regardless of the short axis. If a lymph node has a long axis of 1.1 to 1.5 cm, it should only be considered abnormal if the short axis is more than 1 cm. Lymph nodes ≤ 1 x ≤ 1 cm will not be considered as abnormal for relapse or progressive disease.
  - At least a 50% increase from nadir in the SPD of any previously involved Target Lesions, or in a single involved node or extranodal mass, or the size of other lesions (e.g. splenic or hepatic nodules).
  - To be considered progressive disease, a lymph node or extranodal mass with a diameter of the long or short axis of ≤ 2.0 cm must have increased by at least 0.5 cm; lesions larger than 2.0 cm must have increased by at least 1.0 cm.
  - In the setting of splenomegaly, the splenic length must increase by >50% of the extent of its prior increase from baseline. If no prior splenomegaly, must increase by at least 2.0cm from baseline.
  - New lesions: Regrowth of previously resolved lesions; or a new lymph node > 1.5cm in any axis; or a new extranodal site > 1.0 cm in any axis (new extranodal disease < 1.0 cm in any axis, can be considered progressive disease

if its presence is unequivocal and attributable to lymphoma).
· New or recurrent bone marrow involvement
· Clinical Progressive Disease can be determined using the following criteria:
o ECOG PS of at least 3
o Patient unable to have follow-up radiologic Assessment due to performance status decline
o Symptomatic decline deemed related to metastatic disease or disseminated disease (not toxicity from therapy or concurrent illness).

12.2 Response criteria for acute lymphoblastic leukemia

12.2.1 Complete Remission (CR)

Hematologic complete remission is defined as meeting all of the following response criteria for at least four weeks.

- < 5% blasts in the bone marrow
- Normal maturation of all cellular components in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)
- ANC (absolute neutrophil count) ≥ 1,000/μL
- Platelets ≥ 100,000/μL
- Transfusion independent

In some cases, there may not be a four-week interval between completion of therapy and the pre-transplant disease assessment; in this case, CR should still be reported as the status at transplant, since it represents the "best assessment" prior to HCT. This is an exception to the criteria that CR be durable beyond four weeks. The pre- transplant disease status should not be changed based on early relapse or disease assessment post-transplant.

12.2.2 Complete Remission with Incomplete Hematologic Recovery (CRi)

Hematologic complete remission with incomplete hematologic recovery is defined as meeting all of the following response criteria for at least four weeks:

- < 5% blasts in the bone marrow
- Normal maturation of all cellular components in the bone marrow
- No extramedullary disease (e.g., CNS, soft tissue disease)
- Transfusion independent (Please note, if the physician documents transfusion dependence related to treatment and not the patient's underlying ALL, CRi can be reported)

12.2.3 Primary Induction Failure (PIF)

The patient received treatment for ALL but never achieved CR or CRi at anytime. PIF is not limited by the number of unsuccessful treatments; this disease status only applies to recipients who have never been in CR or CRi.

12.2.4 Relapse (REL)

Relapse is defined as the recurrence of disease after CR, meeting at least one of the following criteria:

- ≥ 5% blasts in the marrow or peripheral blood
- Extramedullary disease
- Disease presence determined by a physician upon clinical assessment

Table 12.2: Response criteria for ALL	
Complete remission (CR)*	Hematologic complete remission is defined as meeting all of the following response criteria for at least four weeks: < 5% blasts in the bone marrow; Normal maturation of all cellular components in the bone marrow; No extramedullary disease (e.g., CNS, soft tissue disease); ANC (absolute neutrophil count) ≥ 1,000/µL; Platelets ≥ 100,000/µL; Transfusion independent
CR with incomplete recovery (CRi)†	Meeting all of the following response criteria for at least four weeks: < 5% blasts in the bone marrow; Normal maturation of all cellular components in the bone marrow; No extramedullary disease (e.g., CNS, soft tissue disease); Transfusion independent
Primary Induction Failure	Never achieved CR or CRi at any time. PIF is not limited by the number of unsuccessful treatments; this disease status only applies to recipients who have never been in CR or CRi.
Relapse¶	Recurrence of disease after CR, meeting at least one of the following criteria: ≥ 5% blasts in the marrow or peripheral blood; Extramedullary disease; Disease presence determined by a physician upon clinical assessment

12.3 Response criteria for chronic lymphocytic leukemia[35] and B-Prolymphocytic leukemia

B-PLL response criteria will align with the criteria for CLL described in this section.

12.3.1 Complete remission requires all of the following criteria (Table 12.3).

1. Peripheral blood lymphocytes (evaluated by blood and differential count) <4 × 10^9/L.
2. Absence of significant lymphadenopathy by physical examination. In clinical trials, a CT scan of the neck, abdomen, pelvis, and thorax is desirable if previously abnormal. Lymph nodes should be <1.5 cm in longest diameter. Once this is

determined, further imaging should not be required until disease progression is apparent by clinical examination or on blood testing.

3. No splenomegaly or hepatomegaly by physical examination. Consensus cutoff for splenomegaly is 13cm.
4. Absence of disease-related constitutional symptoms.
5. Blood counts need to show the following values:
6. Neutrophils $\geq 1.5 \times 10^9$/L.
7. Platelets $\geq 100 \times 10^9$/L.
8. Hemoglobin ≥ 11.0 g/dL (without red blood cell transfusions).

Some patients fulfill all the criteria for a CR (including the marrow examinations described in Section 11), but have a persistent anemia, thrombocytopenia, or neutropenia apparently unrelated to CLL, but related to drug toxicity. These patients should be considered as a different category of remission, **CR with incomplete marrow recovery (CRi)**. For the definition of this category, the marrow evaluation (see Section 11) should be performed with scrutiny and not show any clonal disease infiltrate.

12.3.2 Partial remission

To define a partial remission, at least 2 parameters of group A and 1 parameter of group B need to improve, if previously abnormal (Table 12.3). If only 1 parameter of both groups A and B was abnormal before therapy, only 1 needs to improve. Constitutional symptoms persisting for >1 month should be recorded.

1. A decrease in the number of blood lymphocytes to 50% or less from the value before therapy.
2. Reduction in lymphadenopathy compared with baseline (by cross-sectional imaging scans) as defined by:
    i. A decrease in lymph node size by 50% or more in the sum of the products of the same enlarged lymph nodes selected at baseline as assessed by imaging (up to 6 lymph nodes).
    ii. No increase in any lymph node and no new enlarged lymph node (diameter ≥ 1.5 cm). For small lymph nodes (longest diameter <1.5 cm), an increase <25% is not considered significant.
3. A regression $\geq 50\%$ of the extent of enlargement of the spleen below the costal margin defined by palpation, or normalization in size. When assessed by CT, scan spleen size must have regressed by $\geq 50\%$ in length beyond normal.
4. A regression of $\geq 50\%$ of the extent of enlargement of the liver below the costal margin defined by palpation, or normalization in size. Given the impact of numerous medical conditions, liver size by physical examination or CT scan is not a reliable measure of hepatic involvement by CLL and should only be counted if hepatomegaly is clearly attributable to lymphoid involvement.
5. The blood count should show 1 of the following results:
    i. Platelet counts $>100 \times 10^9$/L or 50% improvement over baseline.
    ii. Hb >11.0 g/dL or 50% improvement over baseline without red blood cell transfusions or erythropoietin support.

12.3.3 Progressive disease

Progressive disease (PD) during or after therapy is characterized by at least 1 of the following, when compared with nadir values (Table 12.3):

1. Lymphadenopathy. Progression of lymphadenopathy is often discovered by physical examination and should be recorded at regular intervals. In CLL, the use of imaging (CT scans) usually does not add much information for the detection of progression or relapse.100 Disease progression occurs if 1 of the following events is observed.
    i. Appearance of any new lesion such as enlarged lymph nodes (≥1.5 cm), splenomegaly, hepatomegaly, or other organ infiltrates. Transient increases of lymph node size during treatment with novel inhibitors may occur and should not be counted as PD.
    ii. An increase by ≥ 50% in greatest determined diameter of any previous site (≥1.5 cm).
2. An increase in the spleen size by ≥ 50% or the de novo appearance of splenomegaly. In the setting of splenomegaly, the splenic length must increase by ≥ 50% of the extent of its prior increase beyond baseline (e.g., a 15-cm spleen must increase to ≥16 cm). If no prior splenomegaly was observed at baseline or if splenomegaly has resolved with treatment, the spleen must increase by at least 2 cm from baseline.
3. An increase in the liver size of ≥ 50% of the extent enlargement of the liver below the costal margin defined by palpation, or the de novo appearance of hepatomegaly. Given the impact of numerous medical conditions, liver size by physical examination or by CT scan is not a reliable measure of hepatic involvement by CLL and should only be counted if hepatomegaly is clearly attributable to lymphoid involvement.
4. An increase in the number of blood lymphocytes by 50% or more with at least 5×10^9/L B lymphocytes. Certain therapies (e.g., kinase inhibitors) may cause lymphocytosis. In the setting of therapy with such agents, an increase in blood lymphocyte count by itself does not uniformly indicate an increased tumor burden, but may reflect redistribution of leukemia cells from lymphoid tissues to the blood. This should be predefined in the protocol of clinical trials for therapies in which redistribution of disease occurs. In such cases, increased lymphocytosis alone is not a sign of treatment failure or PD.
5. Transformation to a more aggressive histology (Richter syndrome or Richter transformation). The diagnosis of Richter transformation should be established by lymph node or other tissue biopsy.
6. Occurrence of cytopenia (neutropenia, anemia, or thrombocytopenia) directly attributable to CLL and unrelated to autoimmune cytopenias.
    a. During therapy, cytopenias cannot be used to define disease progression.
    b. Post-treatment. The progression of any cytopenia (unrelated to autoimmune cytopenia), as documented by a decrease of Hb levels ≥2 g/dL or <10 g/dL, or by a decrease of platelet counts ≥50% or $<100 \times 10^9$/L, which occurs at least 3 months after treatment, defines disease progression, if the marrow biopsy is consistent with the cytopenia resulting from increased marrow infiltration of clonal CLL cells and is not considered a treatment related toxicity.

12.3.4 Stable disease

Patients who have not achieved a CR or a partial remission, and who have not exhibited PD, will be considered to have stable disease (which is equivalent to a nonresponse).

Table 12.3. Response criteria for chronic lymphocytic leukemia[63]

Group	Parameter	CR	PR	PD	SD
A	Lymph nodes	None ≥1.5 cm	Decrease ≥50% (from baseline)*	Increase ≥50% from baseline or fromresponse	Change of −49% to +49%
	Liver and/or spleen size†	Spleen size <13 cm; liver size normal	Decrease ≥50% (from baseline)	Increase ≥50% from baseline or from response	Change of −49% to +49%
	Constitutional symptoms	None	Any	Any	Any
	Circulating lymphocyte count	Normal	Decrease ≥50% frombaseline	Increase ≥50% overbaseline	Change of −49% to +49%
B	Platelet count	≥100 × 10^9/L	≥100 × 10^9/L or increase ≥50% overbaseline	Decrease of ≥50% from baseline secondary to CLL	Change of −49 to +49%
	Hemoglobin	≥11.0 g/dL (untransfused and without erythropoietin)	≥11 g/dLor increase ≥50% overbaseline	Decrease of ≥2 g/dLfrom baseline secondary to CLL	Increase<11.0 g/dLor <50% over baseline, or decrease <2 g/dL
	Marrow	Normocellular, no CLL cells, no B-lymphoid nodules	Presence of CLL cells, or of B-lymphoid nodules, or not done	Increase ofCLL cells by ≥50% on successive biopsies	No change in marrow infiltrate

- For a detailed description of the response parameters, see Section 12.
- ↵* Sum of the products of 6 or fewer lymph nodes (as evaluated by CT scans and physical examination in clinical trials or by physical examination in general practice).
- ↵† Spleen size is considered normal if <13 cm. There is not firmly established international consensus of the size of a normal liver; therefore, liver size should be evaluated by imaging and manual palpation in clinical trials and be recorded according to the definition used in a study protocol.
- CR, complete remission (all of the criteria have to be met); PD, progressive disease (at least 1 of the criteria of group A or group B has to be met); PR, partial remission (for a PR, at least 2 of the parameters of group A *and* 1 parameter of group B need to improve if previously abnormal; if only 1 parameter of both groups A and Bis abnormal before therapy, only 1 needs to improve); SD, stable disease (all of the criteria have to be met; constitutional symptoms alone do not define PD).

12.4 Definitions of Time Periods

12.4.1 Duration of response

This is measured, only in responders, from the documented beginning of response (CR or PR) to the time of relapse.

12.4.2 Disease-free survival

Survival is defined as the date of study entry to the date of death. Disease-free survival is measured from the time of occurrence of disease-free state (e.g. the adjuvant setting following surgery or radiation therapy) or attainment of a complete remission) to disease recurrence or death from lymphoma or acute toxicity of treatment. This definition may be complicated by deaths that occur during the follow-up period that are unrelated to the lymphoma and there is controversy as to whether such deaths should be considered as events or censored at the time of occurrence. Whereas it is often possible to identify those deaths related to the lymphoma, there is the potential for bias in the attribution of deaths.

12.4.3 Disease-specific survival

Disease-specific survival (e.g., lymphoma-specific survival, cause-specific survival) is potentially subject to bias because the exact cause of death is not always easy to ascertain. To minimize the risk of bias, the event should be recorded as death from lymphoma, or from toxicity from the drug. Death from unknown causes should be attributed to the drug. For certain trials, time to next lymphoma treatment may be of interest, defined as time from the end of primary treatment until the initiation of the next therapy.

12.4.4 Progression-free survival

Progression-free Survival (PFS) is defined as the time from entry onto study until lymphoma progression or death from any cause. PFS reflects tumor growth and, therefore, occurs prior to the endpoint of overall survival. In addition, PFS is not confounded by the administration of subsequent therapy. Whether a prolongation of PFS represents direct clinical benefit or a surrogate for clinical benefit depends on the magnitude of the effect and the risk-benefit ratio of the therapy under investigation. Unlike survival, the precise date of progression is generally unknown. It may be defined as the first date of documentation of a new lesion or enlargement of a previous lesion, or the date of the scheduled clinic visit immediately after radiologic assessment has been completed. Where there is missing information, censoring of the data may be defined as the last date at which progression status was adequately assessed or the first date of unscheduled new anti- lymphoma treatment.

12.4.5 Time to progression

Time to progression (TTP) is defined as the time from study entry until lymphoma progression or death due to lymphoma. In TTP, deaths from other causes are censored either at the time of death or at an earlier time of assessment, representing a random pattern of loss from the study. TTP is not as useful as PFS unless the majority of deaths on a study are unrelated to the lymphoma due to the efficacy of the treatment and/or prolonged follow up.

12.4.6 Time to treatment failure

Time to treatment failure (event-free survival) is measured from the time from study entry to any treatment failure including discontinuation of treatment for any reason, such as disease progression, toxicity, patient preference, initiation of new treatment without documented progression, or death. This composite endpoint is generally not encouraged by regulatory agencies because it combines efficacy, toxicity and patient withdrawal.

12.5 Response Review

Responses will be reviewed by the investigator (PI or co-investigator) who is treating the patient at each participating site.

13 CLINICAL TRIAL OVERSIGHT AND MONITORING

13.1 Safety Monitoring

The data and safety monitoring plan will involve the continuous evaluation of safety, data quality and data timeliness. Investigators will conduct continuous review of data and patient safety at their regular Disease Group meetings (at least monthly) and the discussion will be documented in minutes. For each dose level, the Principal Investigator, study coordinator, and statistician, in consultation with treating physicians as appropriate will review all toxicities at a given dose level to inform the model for dose level adjustments. The Principal Investigator of the trial will review toxicities and responses of the trial where applicable at these disease center meetings and determine if the risk/benefit ratio of the trial changes. Frequency and severity of adverse events will be reviewed by the Principal Investigator and compared to what is known about the agent/device from other sources; including published literature, scientific meetings and discussions with sponsors, to determine if the trial should be terminated before completion. Serious adverse events will be reviewed by the OSUCCC Data and Safety Monitoring Committee (DSMC). The Principal Investigator will also submit progress reports that will be reviewed by the committee per the DSMC plan. All reportable SAEs will be reported to the IRB of record as per the policies of the IRB.

Mandatory safety and trial review teleconferences will be scheduled and moderated by the Multi-Center Trial Program (MCTP). All sites involved in the study are expected to have a representative present for every call to review and discuss patients on study and other applicable agenda items. Meeting minutes will be used to document each teleconference. The minutes will be stored in the MCTP protocol files. Teleconferences must minimally be held monthly and may be held more frequently, as needed. For studies closed to accrual with patients expected to remain on long-term treatment and/or follow-up, teleconferences may be extended to occur every two months or quarterly. Decreasing frequency of teleconferences requires OSU PI and MCTP approval.

13.2 Data Safety Monitoring Committee (DSMC)

The data and safety monitoring plan will involve the continuous evaluation of safety, data quality and data timeliness. Investigators will conduct continuous review of data and patient safety at their

regular Disease Group meetings (at least monthly) and the discussion will be documented in minutes. For each dose level, the Principal Investigator, study coordinator, and statistician, in consultation with treating physicians as appropriate will review all toxicities at a given dose level to inform the model for dose level adjustments. The Principal Investigator of the trial will review toxicities and responses of the trial where applicable at these disease center meetings and determine if the risk/benefit ratio of the trial changes. Frequency and severity of adverse events will be reviewed by the Principal Investigator and compared to what is known about the agent/device from other sources; including published literature, scientific meetings and discussions with sponsors, to determine if the trial should be terminated before completion. Serious adverse events will be reviewed by the OSUCCC Data and Safety Monitoring Committee (DSMC). The Principal Investigator will also submit progress reports that will be reviewed by the committee per the DSMC plan. All reportable SAEs will be reported to the IRB of record as per the policies of the IRB.

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13.2.1 Data Submission

The study will be managed per the Multi-Center Trial Program (MCTP) policies. Subsite data must be submitted to the MCTP as outlined in the protocol-specific monitoring plan. The protocol-specific monitoring plan will be provided by the MCTP to external participating sites prior to site activation. Data will be submitted using case report forms and the Data Submission Form cover sheet (refer to Supplemental Forms Document) supplied by the MCTP. Access to the OSU OnCore database will be provided to external participating sites for direct electronic data entry. All data submitted must be accompanied by supporting source documents, where applicable and as outlined in the protocol-specific monitoring plan.

13.2.2 Protocol Deviations

Any protocol deviation by an Investigator at a subsite must be reported to the trial Sponsor (OSU) and IRB. If the Sponsor determines the deviation impacts the usefulness or quality of the study data, the Sponsor will report this to the FDA in the next annual report.

13.3 Study Monitoring

The Local Site Investigators will permit study-related monitoring visits by representatives of the sponsor or designees, and regulatory inspections(s) (e.g., FDA) to ensure proper conduct of the study and compliance with all FDA safety reporting requirements. Access will be provided to source documents, to CRFs, and to all other study documents.

This protocol will be monitored remotely by The Ohio State University Clinical Trials Office. Monitoring will occur after the third participant reaches Day +100, then annually until the protocol is closed when the study will undergo a final monitoring visit. The frequency of monitoring may

be increased if deemed necessary to assure compliance and protect participant safety.
The following information will be reviewed at each monitoring visit:

- Informed Consent
- Regulatory Documentation
- Eligibility Criteria
- Protocol Adherence
- Adverse Events
- Printed Electronic Source Documentation

13.3.1 Auditing

As the study sponsor, The Ohio State University Comprehensive Cancer Center (OSUCCC) will audit each site as per OSU policies. Audits will be performed by the OSUCCC Clinical Research Audit Team. For sites with an auditing mechanism in place that are able to share documentation of their auditing standards and processes followed, an agreement may be requested for the site to perform local auditing and provide formal audit reports to the OSUCCC Multi-Center Trial Program (MCTP) and the Quality Assurance Oversight Committee.

13.3.2 Confidentiality and Privacy

All data and records generated during this study will be kept confidential in accordance with Institutional policies and HIPAA on subject privacy. The Principal Investigator and other site personnel will not use data or PHI for any purpose other than conduction of the study. All PHI used will be de-identified and coded by the Principal investigator. The code will be kept in a password protected computer file. PHI will be disclosed when required for audit by regulatory agencies. Information from medical records and from the procedures, interviews and tests that are part of this research will be collected. We will do our best to keep personal information private and confidential. However, we cannot guarantee absolute confidentiality. Personal information may be disclosed if required by law. The results of this study may be shown at meetings or published in journals to inform other doctors and health professionals. Subject identity will be kept private in any publication or presentation about the study. People and organizations that may inspect and/or copy research records to assure the quality of the data and to analyze the data include:

- Medical staff who are directly or indirectly involved in care related to this research
- People who oversee or evaluate research and care activities at Ohio State University and Nationwide Children's Hospital
- People from agencies and organizations that perform independent accreditation and oversight of research
- The Food and Drug Administration

14 STATISTICAL CONSIDERATIONS

14.1 Sample size and analysis of primary endpoint.

With this type of phase I study design, the exact number of patients needed to complete the study is unknown, as it depends on the number of cohorts required to reach the MTD. A maximum of 18 patients per cohort can theoretically participate in the dose escalation, based on 3 dose levels, with a maximum of 6 patients at each dose level. Table 14.1 gives the probabilities of escalating the dose level under a true but unknown underlying rate of DLT. At a true DLT rate of 20%, the chance of escalating to the next dose level is 71% and of establishing the lower dose level as MTD is 29%. At a true DLT rate of 50%, the probability of escalating to the next dose level is 17%, and of establishing the prior dose level as the MTD is 83%.

Table 14.1: Probability of escalating dose levels

	True underlying DLT rate				
	0.1	0.2	0.3	0.4	0.5
Probability of escalating to next dose level	0.91	0.71	0.49	0.32	0.17
Probability of not escalating and establishing prior dose level as MTD	0.09	0.29	0.51	0.68	0.83

14.2 Statistical analysis of secondary endpoints

Secondary endpoints will be evaluated with descriptive statistics for the frequencies of adverse events of different grades, with proportions used to describe response rates (complete and overall). Survival rates will be calculated using Kaplan Meier method, comparisons between groups will be done using the log rank test.

14.3 Correlative studies

The results of correlative studies conducted in this trial will be analyzed with descriptive statistics, including evaluations of median, minimum and peak values. Area under the curve (AUC) will be used as a measure of cytokine secretion as well as of CAR-T cell expansion and persistence. Comparisons will be done between responding and non-responding patients using the Mann – Whitney test for quantitative variables and Fisher's exact test for categorical variables. The Wilcoxon test will be used to evaluate the changes in cytokine concentrations, CAR-T cell expansion and changes in T cell phenotype over time. While the sample size may not allow for achievement of statistical significance in association tests, we will evaluate the presence of associations between cytokine serum concentrations and CAR-T cell expansion (measured in absolute number changes, percentage changes and AUC) as well as with presence or absence or response and adverse events. Correlations between quantitative variables will be done with Spearman's correlation test, whereas correlation between categorical variables will be done using Fisher's exact test. Statistical analysis will be done using XLSTAT ® and R Software.

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16 APPENDICES

16.1 APPENDIX 1. PERFORMANCE STATUS CRITERIA FOR ADULTS

ADULTS (individuals 16 years and older)

ECOG Performance Status Scale			
Grade	Description	Percent	Description
0	Normal activity. Full active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% if waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed > 50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead

16.2 APPENDIX 2. PERFORMANCE STATUS CRITERIA FOR CHILDREN

CHILDREN (individuals younger than 16 years)

Lansky Performance Scale	Description
100	Fully active
90	Minor restriction in physically strenuous play
80	Restricted in strenuous play, tires more easily, otherwise active
70	Both greater restrictions of, and less time spent in active play
60	Ambulatory up to 50% of time, limited active play with assistance / supervision
50	Considerable assistance required for any active play, fully able to engage in quiet play
40	Able to initiate quiet activities
30	Needs considerable assistance for quiet activity
20	Limited to very passive activity initiated by others (e.g. TV)
10	Completely disabled, not even passive play

16.3 APPENDIX 3. EXAMPLE CERTIFICATE OF ANALYSIS

FRESH CAR T-CELL CERTIFICATE OF ANALYSIS

PROTOCOL # Enter ID. **RECIPIENT Name:** Enter name or N/A. Date of Birth: Enter MM/DD/YYYY or N/A.
Study ID #: Enter ID. Weight: ###.# kg MRN: Enter MRN or N/A.

PRODUCT **DIN:** Enter DIN. **Product Code:** enter code **Division(s):** N/A for fresh dose
Container: Select. **Container Contents:** ## mL, ## x10^6 CD19CAR+ T-cells
Collected: MM/DD/YYYY 00:00 Select **Harvested:** MM/DD/YYYY 00:00 Select **Expiration:** MM/DD/YYYY 00:00 Select
Final Formulation: CAR T-cells in PlasmaLyte-A, 0.5% (w/v) HSA Completed By (initials):

Test	Test Method *(sample, if not final formulation)*	Criteria	Result	Passes / Fails
Viability	Trypan Blue	≥ 70% viable	##% viable	Select
Purity *Reported as % of viable CD45+ cells*	Surface Markers by Flow Cytometry	≥ 90% CD3+ T cells	##% T cells	Select
		info only: % CD19+	##% CD19+	N/A
		info only: % CD14+	##% CD14+	N/A
		info only: % CD56/16+	##% CD56/16+	N/A
CAR T Transduction Efficiency *Reported as % of viable CD45+ cells*	Surface Markers by Flow Cytometry	≥ 10% CD19 CAR+	##% CD19 CAR+	Select
		info only: % CD22 CAR+	##% CD22 CAR+	N/A
CD19/CD20/CD22 CAR T cell dose	Calculated	**Dose level met**: [X]=assigned cohort ☐ -1: 1x10^5 cells/kg ☐ 1: 5x10^5 cells/kg ☐ 2: 1x10^6 cells/kg ☐ 3: 2x10^6 cells/kg	Select ## x10^6 CAR T cells /kg	Select
Microbial Contamination	14-Day Blood Culture – Aerobic Bactec™	Pending, day 0	Select, day 0	Select
	14-Day Blood Culture – Anaerobic Bactec™		Select, day 0	Select
	Gram Stain	No organisms seen	Enter text.	Select
	Visual Inspection	No evidence of contamination	Enter text.	Select
Replication-Competent Lentivirus	PCR *(day prior to harvest)*	Negative	Select	Select
Mycoplasma	PCR *(day prior to harvest)*	Negative	Select	Select
Endotoxin	Endosafe™ Kinetic Colorimetric	≤ 5 EU/kg recipient weight per hour	## EU/kg/hr	Select

Records reviewed for process control & accuracy. **Product meets all release criteria?** ☐ **Yes** → ☐ Available for Distribution
☐ **NO** → ☐ Exceptional Release Approved ☐ QUARANTINE ☐ Label as "Not for Infusion" ☐ Available for Pre-Release Distribution
APPROVED BY:

Name _____ Signature _____ Title _____ Date / Time _____

Post-Distribution - *Enter results by hand on COA signed at release*

Microbial Contamination	Blood Culture – Aerobic Bactec™	No Growth Final		
	Blood Culture – Anaerobic Bactec™			

Quality Management Team Final Batch Record Review: Final records reviewed for process control & accuracy.
Product meets post-distribution criteria? ☐ ***N/A*** ☐ **Yes** ☐ **NO** → ☐ Complete all notifications

Name / Title: Manufacturing _____ Signature _____ Date _____

Name / Title: Quality Assurance _____ Signature _____ Date _____

COMMENTS:

FORM 357.1, *December 2021* Ohio State University Wexner Medical Center
Page 1 of 1 *Information in gray pre-approved for each protocol & form locked.* The James Cell Therapy Laboratory

CRYOPRESERVED CAR T-CELL CERTIFICATE OF ANALYSIS

PROTOCOL # Enter text. **RECIPIENT Name:** Enter name or N/A. **Date of Birth:** Enter MM/DD/YYYY or N/A.
Study ID #: Enter ID. **Weight:** ###.# kg **MRN:** Enter MRN or N/A.

PRODUCT **DIN:** Enter DIN. **Product Code:** enter code **Division(s):** #s or range
Container: Select ** ## Containers/dose **Container Contents:** ## mL, ## x10^6 CD19CAR+ T-cells
Collected: MM/DD/YYYY 00:00 Select **Harvested:** MM/DD/YYYY 00:00 Select **Expiration: 30 minutes after thaw**
Final Formulation: CAR T-cells in PlasmaLyte-A, 5% DMSO, 12.5% (w/v) HSA **Completed By (initials):**

Test	Test Method (sample*, if not final formulation)	Criteria	Result	Passes / Fails
Viability	Trypan Blue (post-harvest, pre-DMSO addition)	≥ 70% viable	##% viable	Select
Purity Reported as % of viable CD45+ cells	Surface Markers by Flow Cytometry (post-harvest, pre-DMSO addition)	≥ 90% CD3+ T cells	##% T cells	Select
		info only: % CD19+	##% CD19+	N/A
		info only: % CD14+	##% CD14+	N/A
		info only: % CD56/16+	##% CD56/16+	N/A
CAR T Transduction Efficiency Reported as % of viable CD45+ cells	Surface Markers by Flow Cytometry (post-harvest, pre-DMSO addition)	≥ 10% CD19 CAR+	##% CD19 CAR+	Select
		info only: % CD22 CAR+	##% CD22 CAR+	N/A
CD19/CD20/CD22 CAR T cell dose	Calculated (post-harvest, pre-DMSO addition)	**Dose level met** ☒=assigned cohort ☐ -1: 1x10^5 cells/kg ☐ 1: 5x10^5 cells/kg ☐ 2: 1x10^6 cells/kg ☐ 3: 2x10^6 cells/kg	Select ## x10^6 CAR T cells /kg	Select
Microbial Contamination	14-Day Blood Culture – Aerobic Bactec™	No Growth at 14 days or Negative to date	Select, day ##	Select
	14-Day Blood Culture – Anaerobic Bactec™		Select, day ##	Select
	Gram Stain (post-harvest, pre-DMSO addition)	No Organisms Seen	Enter text.	Select
	Visual Inspection	No evidence of contamination	Enter text.	Select
Replication-Competent Lentivirus	PCR (day prior to harvest)	Negative	Select	Select
Mycoplasma	PCR (day prior to harvest)	Negative	Select	Select
Endotoxin	Endosafe™ Kinetic Colorimetric	≤ 5 EU/kg recipient weight per hour	## EU/kg/hr	Select

Quality Management Team Review: Product meets all release criteria? ☐ **Yes** → ☐ Available for Distribution
☐ **NO** → ☐ Exceptional Release Approved ☐ QUARANTINE ☐ Label as "Not for Infusion" ☐ Available for Pre-Release Distribution
APPROVED BY:

Name	Signature	Title	Date / Time

Post-Distribution - *Enter results by hand on the COA signed at release OR Enter N/A below*

Microbial Contamination	Blood Culture – Aerobic Bactec™	No Growth Final		
	Blood Culture – Anaerobic Bactec™			

Quality Management Team Final Batch Record Review: Final records reviewed for process control & accuracy.
Product meets post-distribution criteria? ☐ ***N/A*** ☐ **Yes** ☐ **NO** → ☐ Complete all notifications

Name / Title: Manufacturing	Signature	Date
Name / Title: Quality Assurance	Signature	Date

COMMENTS:

16.4 APPENDIX 4. MANAGEMENT OF ADVERSE EVENTS FOLLOWING IMMUNE EFFECTOR CELL THERAPY

The James

Plan of Care: CAR-T and Other Immune Effector Cell Therapy, Management of patients undergoing Therapy

Applicable Program: ***BMT (Blood and Marrow Transplant)***

SOP#: 63.08

1. SCOPE:

1.1. To provide guidance on the management of toxicity and complications associated with CAR-T therapy in adult patients. The same guidelines applies to other adoptive cellular (immune effector cell) therapy. Patients enrolled on clinical trials will be managed per guidance of the trial protocol. These guidelines do NOT include management of CRS with haploidentical HCT or COVID-19 disease.

2. OBJECTIVES:

2.1. Define toxicities associated with adoptive cellular therapy including CAR-T therapy.

2.2. Provide guidance for management of these toxicities.

3. DEFINITION:

3.1. FACT-competent provider: A physician or APP who has completed the FACT competency form and FACT-required continuing education requirements, and has completed REMS training.

4. BACKGROUND:

4.1. Upon infusion of adoptive cellular products, cytokines are released into the circulation resulting in systemic effects often manifested as clinical toxicity. In most patients, the symptoms are mild to moderate in severity. Some patients may experience severe, life-threatening complications often encountered with high disease burden.

4.2. Cytokine release syndrome (CRS) is an oncologic emergency, and special precautions must be taken to prevent life-threatening consequences. Other complications that may also be life-threatening are neurological toxicities (with/without CRS), HLH/MAS (typically occur with CRS), tumor lysis syndrome (with risk of acute kidney injury) and prolonged cytopenia.

4.3. Infectious complications may occur in the context of febrile neutropenia, steroid therapy, or hypogammaglobulinemia.

5. PLAN OF CARE:

5.1. Preparation for CAR-T therapy

5.1.1. Patients will have a central venous access using double or triple lumen catheter (e.g. PICC line).

5.1.2. CSF testing may be considered if clinical suspicion of CNS disease involvement.

5.1.3. Baseline MRI brain may be considered if suspicious neurological deficit.

5.2. Lymphodepleting Chemotherapy

5.2.1. Patients may receive lymphodepletion regimen as outpatient or inpatient.

5.2.2. Lymphodepletion chemotherapy may be omitted in case of leukopenia/lymphopenia per the manufacturer's guidance in the package insert. This must be verified/discussed with the clinical pharmacist.

5.3. Prophylactic measures during CAR-T therapy

5.3.1. Tumor lysis syndrome (TLS) prophylaxis (allopurinol and intravenous fluid hydration) will be used for patients with high disease burden.

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5.3.2. Seizure prophylaxis will not be routinely used, but may be considered in patients with prior CNS disease or with history of seizure in the past.

5.3.3. Antibiotic prophylaxis will be administered per section of "infection prevention" below.

5.4. Antiviral Prophylaxis

5.4.1. For patients who are HBV or HCV positive, management will follow BMT 19 Infection Prophylaxis and Management of HCT.

5.4.2. Referral to Hepatology is recommended for patients with active disease. Suppressive therapy for HBV (e.g. entecavir) is recommended (before starting lymphodepletion) for patients who are HBV positive.

5.5. Monitoring during therapy

5.5.1. Once lymphodepletion chemotherapy is started, patients will be monitored (either in the inpatient or outpatient setting) by a FACT-competent provider.

5.5.2. Outpatient CAR-T therapy requires daily clinical assessment (post-infusion) to monitor for acute toxicity, as detailed below, for at least 7 days and less frequently thereafter.

5.5.3. Patients may be hospitalized (on the day of cellular product infusion) for at least 7 days to monitor for acute toxicity as detailed below.

5.5.4. During hospitalization, patients will be evaluated daily by the FACT-competent provider and at least every 12 hours by the RN.

5.5.4.1. More frequent monitoring will be implemented for patients with hemodynamic or neurological deficit.

5.5.5. Clinical assessment will include vital signs, pulse oximetry, and mental status.

5.5.6. Assessment and grading of CRS and neurotoxicity will be done using specific tools as discussed below.

5.5.7. Cardiac monitoring is recommended for patients with CRS grade ≥2 (risk of arrhythmia).

5.5.8. Early consultation of Medical Intensive Care Unit team is recommended when patient becomes hemodynamically or neurologically unstable. Assisted ventilation may be indicated with hypoxemia or significant mental status alteration.

5.6. Laboratory Monitoring

5.6.1. Lab workup during the first 7 days post-infusion are as follows. Subsequent lab monitoring will be determined as clinically indicated.

5.6.1.1. CBC (daily)

5.6.1.2. Basic metabolic profile (daily): electrolytes and renal function tests

5.6.1.3. Hepatic function test (3 times a week)

5.6.1.4. Coagulation profile (3 times a week)

5.6.1.5. Ferritin, LDH and CRP (daily)

5.6.1.6. IgG level will be checked once at day +7 as a baseline. It may be repeated (weekly) if recurrent or prolonged infections for possible replacement (as discussed later)

5.6.1.7. Other testing such as lactate and pro-calcitonin may be considered if suspecting sepsis

5.6.1.8. Pro-BNP is considered if suspecting cardiac failure

5.7. Discharge planning

5.7.1. Upon discharge, patient and caregiver will be instructed to promptly report any symptoms on a 24/7 for 4 weeks following therapy.

5.7.2. Patients are instructed not to drive or operate heavy equipment for at least 8 weeks post-infusion (risk duration of neurotoxicity).

5.8. Ambulatory Care (post-discharge)

5.8.1. Patients will be evaluated in the clinic once or twice a week.

5.8.2. Lab work up may be monitored more often if clinically indicated (e.g. transfusion requirement) until day 28 post-infusion.

5.8.3. Ambulatory visits may then be every 2-4 weeks (until disease assessment) or more frequently if clinically indicated.

5.9. Disease Assessment:

5.9.1. PET scan/CT scan (or other disease assessment testing) will be obtained at day +60 post-infusion.

5.9.2. Earlier testing (at day +30) may be obtained if suspected disease progression.

5.9.3. Later testing at day +90 may be obtained if clinical evidence of disease response.

6. CYTOKINE RELEASE SYNDROME (CRS)

6.1. DIAGNOSIS:

6.1.1. CRS often occurs within 3 days of infusion (delayed onset may be up to 7 weeks) and lasts for about 7 days (may last up to 8 weeks).

6.1.2. It manifests as fever (may be associated with malaise, chills or arthralgia), with/without hypotension or hypoxemia.

6.1.3. The cardinal features of CRS are defined as follows (when not attributable to other causes):

6.1.3.1. **Fever** (temperature ≥38C/100.4F).

6.1.3.2. **Hypotension** (MAP<65 or SBP<90 with symptoms).

6.1.3.3. **Hypoxia** (O2 saturation ≤90% on room air).

6.1.4. Other organ toxicity may be encountered with CRS as follows:

6.1.4.1. Cardiac: tachyarrhythmia's (e.g. atrial fibrillation, ventricular tachycardia), low LVEF.

6.1.4.2. Respiratory: pleural effusion, pulmonary edema.

6.1.4.3. Renal: Acute Kidney Injury (AKI)

6.1.4.4. Hepatic: increased ALT/AST or bilirubin

6.1.4.5. Gastrointestinal: nausea, vomiting, diarrhea

6.1.4.6. Skin: Rash

6.2. GRADING OF CRS:

6.2.1. The following grading system (ASTCT/NCCN) is used. Organ toxicity may be graded independently by CTCAE version 5.0.

CRS Grade[1]	Manifestation(s)
1	**Fever**[2] = Temp ≥38-C (100.4-F)
2	**Fever** with **Hypotension** responsive to IVF (not requiring vasopressor). **OR** **Hypoxemia** requiring low-flow nasal cannula (≤6 L/minute), or blow-by (used in children).
3	**Fever** with **Hypotension** requiring single vasopressor with or without vasopressin) **OR** **Hypoxemia** high-flow nasal cannula (>6 L/minute), facemask, or Venturi mask.
4	**Fever** with **Hypotension** requiring multiple vasopressors (excluding vasopressin) **OR** **Hypoxemia** requiring positive pressure ventilation (e.g. CPAP, BiPAP, and mech vent).

[1]CRS grade is determined by the more severe event. For example, a patient with a temperature of ≥39.5-C (103.1-F), hypotension requiring one vasopressor, and hypoxia requiring low-flow nasal cannula is classified as grade three CRS.

[2]Fever is defined as temperature ≥38-C (100.4-F) not attributable to any other cause. In patients with CRS receiving antipyretics or anticytokine therapy such as tocilizumab or steroids, fever is no longer required to grade subsequent CRS severity. In this case, CRS will be graded hypotension and/or hypoxia criteria.

6.3. MANAGEMENT OF CRS:

6.3.1. CRS management will be according to the following table. The use Tocilizumab or steroid use must be **approved by attending physician before administration.**

6.4. Cytokine Release Management Table

CRS Grade	Manifestation(s)	Management
1	**Fever** = Temp ≥38-C (100.4-F)	• Assess for infection • Start antibiotics if neutropenic and infection suspected • Antipyretics (avoid NSAID) • Limit use of meperidine • G-CSF (not GM-CSF) may be used after only one week (post-infusion) for complicated febrile neutropenia **(only for grade 1 CRS).**[3] **Specific therapy** ◊ Consider **tocilizumab.**[4] ◊ If no response to tocilizumab (x1 dose), may consider **dexamethasone 10 mg x1 dose** (in particular with Yescarta).
2	**Fever** with **Hypotension** responsive to IVF (not requiring vasopressor). **OR** **Hypoxemia** requiring low-flow nasal cannula (≤6 L/minute), or blow-by (used in children).	**Fever:** as grade 1. **Hypotension** • IV fluid bolus up. If no response after 2L, start vasopressor (thus becomes grade 3). • Cardiac monitor • Assess for infection • EKG, troponins, pro-BNP/echo (if clinically indicated). **Hypoxemia** • Supplemental Oxygen • Chest imaging. **Specific therapy** ◊ Tocilizumab[4] will be given. ◊ Dexamethasone 10 mg (x1 dose) *may be considered* (for example, in elderly, high tumor burden, very high baseline ferritin/CRP levels). ◊ If no response (persistent hypotension) after 1-2 doses of tocilizumab → Dexamethasone 10 mg IV every 6 hours and taper.[5]
3	**Fever** with **Hypotension** requiring single vasopressor with or without vasopressin) **OR** **Hypoxemia** high-flow nasal cannula (>6 L/minute), facemask, or Venturi mask.	**Fever:** as grade 1. **Hypotension** • As grade 2 • Vasopressor (see appendix for definitions). • 2D echo is recommended. • Consider ICU transfer. **Hypoxemia** • As grade 2 **Specific therapy** ◊ Tocilizumab[4] will be given, **plus** ◊ Dexamethasone 10 mg IV every 6 hours and taper.[5]

		◊ If no response (after 7 days) → manage as grade 4.
4	**Fever** with **Hypotension** requiring multiple vasopressors (excluding vasopressin) **OR** **Hypoxemia** requiring positive pressure ventilation (e.g. CPAP, BiPAP, and mechanical ventilation).	**Fever:** as grade 1. **Hypotension** • As grade 2 • Vasopressors (see appendix for definitions). • ICU transfer. **Hypoxemia** • Positive pressure ventilation (per ICU team). **Specific therapy** ◊ Tocilizumab[4] will be given, **plus** ◊ Dexamethasone 10 mg IV every 6 hours and taper.[5] ◊ If no response → methylprednisolone IV 1,000 mg/day and taper.[5]

[3]GM-CSF is contraindicated after CAR-T therapy. However, after at least one week post-infusion and in the absence of grade 2 or more CRS, G-CSF may be cautiously considered for prolonged neutropenia (>14 days) or complicated febrile neutropenia (per NCCN guidelines of Treatment of Cancer-Related Infections) e.g. with progressive sepsis with bacteremia.

[4]Tocilizumab is dosed as 8 mg/kg (maximum dose is 800 mg/dose - see appendix for dose rounding), may repeat every 8 hours if no response (maximum of 3 doses). All doses must be approved by attending physician.

[5]Steroid therapy and taper is suggested as follow:

- Dexamethasone 10 mg IV every 6 hours for 1-3 days (or longer if no response), then taper to every 8 hours for 1-3 days, then every 12 hours for 1-3 days, then every 24 hours or stop. Longer duration (slower taper with/without re-escalation) may be warranted in non-responding cases.
- Methylprednisolone IV 1,000 mg/day for 1-3 days, then taper to 250 mg every 12 hours for 1-2 days, then 125 mg every 12 hours for 1-2 days, then 60 mg every 12 hours for 1-2 days (or until resolution of toxicity), then stop.

7. NEUROTOXICITY

7.1. DIAGNOSIS:

7.1.1. Neurological toxicity typically starts within 4-10 days and lasts for 14-17 days after product infusion. Late onset may occur as late as 3rd or 4th week post infusion and in rare cases up to 6 weeks.

7.2. MANIFESTATIONS:

7.2.1. It often manifests as encephalopathy or focal deficit. Fatal cases of cerebral edema have occurred. The following list is the commonly seen manifestations.

7.2.1.1. Encephalopathy (confusion, dizziness, drowsiness, lethargy, delirium, or coma).

7.2.1.2. Psychiatric disturbances (anxiety, agitation/irritability, psychosis, insomnia)

7.2.1.3. Headache.

7.2.1.4. Focal deficit: aphasia, limb paralysis, or incontinence.

7.2.1.5. Seizures or tremors.

7.2.1.6. Autonomic neuropathy.

7.3. GRADING:

7.3.1. Grading of neurological toxicity is implemented as recommended by ASTCT in a 2-step fashion as follows:

7.3.1.1. ICE score (mental status assessment tool): 0-10 points.

7.3.1.2. ICANS grading (overall neurotoxicity grading).

7.4. Immune Effector Cell-Associated Encephalopathy (ICE) Assessment Tool

Topic	Assessment	Score
1. Orientation	Year, month, city, and hospital	4
2. Cognition	**3 objects** pointed out and named within the room	3
3. Comprehension	Ability to follow simple command: e.g. "show me 2 fingers".	1
4. Attention	**Count** backward from 100 by 10's.	1
5. Motor function	***Write*** a standard sentence (preferred to be the same every time for comparison).	1

7.5. ASTCT Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS) Consensus grading.[1]

Manifestation	Grade 1	Grade 2	Grade 3	Grade 4
ICE score[2]	7-9	3-6	0-2	0 (patient is unarousable/coma)
Altered[3] consciousness	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable (coma) or requires vigorous or repetitive tactile stimuli to arouse (stupor).
Seizure	N/A	N/A	• Focal/ generalized seizure, OR • Nonconvulsive seizures (on EEG) Either resolves rapidly with intervention.	• Life-threatening or prolonged seizure (>5 min), OR • Repetitive clinical or electrical seizures without return to baseline in between (status epilepticus).
Motor findings	N/A	N/A	N/A	Focal motor weakness (e.g. hemiparesis or paraparesis).
High ICP (cerebral edema)	N/A	N/A	Focal cerebral edema on neuroimaging.	Any of the following[4] • Diffuse cerebral edema • Decerebrate/decorticate posturing • Cranial nerve VI palsy • Papilledema • Cushing's triad

[1]ICANS grade is determined by the most severe event (not attributable to any other cause). For example, a patient with an ICE score of 3 who has a generalized seizure is classified as grade 3 ICANS.

[2]ICE score of 0 may be classified as grade 3 ICANS if awake with global aphasia, or grade 4 ICANS if unarousable (comatosed).

[3]Altered level of consciousness should be attributable to no other cause (e.g. no sedating medication).

[4]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.

[5]Other signs and symptoms of neurotoxicity such as headache, tremors, myoclonus, and hallucination are NOT included in ICANS grading, but directed therapy may be warranted.

7.6. NEUROTOXICITY MANAGEMENT

7.6.1. The following table describes the management of neurotoxicity noting the following:

7.6.2. **Steroid therapy/taper:** steroid therapy is continued until **neurotoxicity is ≤ grade 1**. Then steroid is tapered as per guidelines for CRS (see before). If toxicity grade worsens during taper, return to previous dose level and consider slower taper per clinical judgment.

7.6.3. Off-label use of expensive therapy (e.g. siltuximab and anakinra) must be approved by the program director before requesting approval from pharmacy and hospital administration

7.6.4. Anti-seizure medication (e.g. Keppra) should be continued for total of 30 days.

7.6.5. Caution when using medications that can cause CNS depression.

7.6.6. Patients with encephalopathy needs "aspiration precaution" and maintenance IV hydration.

7.7. Neurotoxicity Treatment Recommendations Table

Grade	Treatment Recommendation
1	• Levetiracetam (Keppra®) prophylaxis (500 mg PO/IV q 12 hours). • Consider neurology consult.
2	• Levetiracetam (Keppra®) prophylaxis (as above) • Brain imaging (MRI preferred over CT scan). • EEG monitoring. • Neurology Consult. • Consider Diagnostic Lumbar Puncture (unless clinically contraindicated). • Consider transfer to MICU (in particular if CRS grade ≥2). • **Steroid therapy:*** ○ Dexamethasone 10 mg IV x1 dose. And if persistent grade 2, then ○ Dexamethasone 10 mg IV every 6 hours, then taper.[1] ○ If no improvement or progression in 48-72 hours, consider therapies listed for grad 4.
3	• Levetiracetam (Keppra®) prophylaxis (as above), or therapy (if applicable). • Brain imaging (MRI preferred), and may repeat every 2-3 days if persistent grade ≥ toxicity. • EEG monitoring. • Neurology Consult. • Diagnostic Lumbar Puncture (unless clinically contraindicated). • Transfer to MICU is recommended. • **Steroid therapy:*** ○ Dexamethasone 10 mg IV every 6 hours, then taper.[1] ○ If no improvement or progression in 48-72 hours consider therapies listed for grad 4.
4	• Levetiracetam (Keppra®) prophylaxis (as above), or therapy (if applicable).

- Brain imaging (MRI preferred), and may repeat every 2-3 days if persistent grade ≥ toxicity.
- EEG monitoring.
- Neurology Consult.
- Diagnostic Lumbar Puncture (unless clinically contraindicated).
- Transfer to MICU (consider mechanical ventilation for airway protection).
- **Steroid therapy:**[1]
  - Solumedrol 1 gm IV daily **(for at least 3 days)** until neurotoxicity is ≤ G1, the taper.[1]
- If grade 4 neurotoxicity persists x ≥72 hours, consider the following:
  - LP with IT triple therapy (MTX 15 mg, Cytarabine 40 mg and Hydrocortisone 50 mg).
  - Siltuximab 11mg/kg x 1 dose, may repeat in 10 days.
  - Anakinra.
  - ATG (may eliminate CAR-T cells).

[1]Steroid taper will follow the same guidelines as with the CRS (see before).

8. HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS (HLH)/MACROPHAGE ACTIVATION SYNDROME (MAS)

8.1. ASSOCIATION WITH CRS: MAS/HLH is typically associated with CRS (often high grade). This syndrome is caused by the excessive activation of T cells and macrophages.

8.2. CLINICAL MANIFESTATIONS: HLH/MAS is a febrile illness often associated with cytopenia and multiple organ dysfunction.

8.3. CYTOPENIA is defined as at least 2 of the following: Hemoglobin <9 gm/dl, ANC <1,000/mm^3, or platelet count <100,000/mm^3.

8.4. COAGULOPATHY: abnormal coagulation profile may occur with HLH/MAS. This will include high PT/PTT, D-dimer and **low fibrinogen.** Disseminated intravascular coagulation (DIC) may also occur.

8.5. OTHER LABORATORY ABNORMALITIES: elevated ferritin, triglycerides or soluble Il-2 receptor (CD25).

8.6. DIAGNOSIS: Diagnostic Criteria for CAR-T-related HLH/MAS are as follows:

8.6.1. Clinical diagnosis: Rapidly rising serum ferritin level (>5,000 ng/ml) with "cytopenia" (during CRS) especially if associated with any of the following (grading per CTCAE v 5.0):

8.6.1.1. Grade ≥3 increase in serum bilirubin, AST, ALT.

8.6.1.2. Grade ≥3 oliguria or increase of serum creatinine.

8.6.1.3. Grade ≥3 pulmonary edema

8.6.1.4. Pathological diagnosis: Presence of hemophagocytosis in bone marrow or organs based on histopathological assessment of cell morphology and/or CD68 immunohistochemistry.

8.7. MANAGEMENT:

8.7.1. Established cases will have daily monitoring of blood ferritin, LDH, fibrinogen, transaminases, bilirubin, and serum creatinine levels until resolved or stabilized.

8.7.2. Grade ≥ 3 organ toxicity will be managed with tocilizumab (per CRS algorithm) ***plus*** steroids (e.g. Dexamethasone 10 mg IV every 6 hours and tapered as per CRS guidelines).

8.7.3. If no improvement after 48 hours, consider the following:

8.7.3.1. Intrathecal cytarabine (100 mg), and hydrocortisone (50-100 mg) if associated neurotoxicity.

8.7.3.2. **Anakinra** (second line after steroid failure) with or without steroid escalation to **Methylprednisolone** 1 gm daily (then taper as per CRS guidelines).

8.7.3.3. Etoposide 75-100 mg/m^2 IV and it may be repeated after 4-7 days (if clinically indicated). *This may be used ONLY after discussion with the primary physician who prescribed the CAR T therapy*. Etoposide therapy will likely eliminate the CAR T cells, thus reserved for life-threatening situations after failure of both anakinra and steroid.

9. TUMOR LYSIS SYNDROME (TLS)

9.1. This will be managed per Acute Leukemia Plan of Care 10 – Management of Tumor Lysis Syndrome.

10. CYTOPENIA

10.1. Cytopenia is common after CAR-T therapy. This may include anemia, neutropenia, and/or thrombocytopenia. It may be prolonged and severe (grade 3-4).

10.2. Etiology of cytopenia in this setting includes preceding lymphodepletion and also myelosuppressive cytokine release following CAR-T therapy. Notably, cytopenia may occur without lymphodepletion therapy.

10.3. GM-CSF is NOT recommended in the setting of CRS.

10.4. G-CSF may be used for prolonged cytopenia (>2 weeks post infusion) if there is no evidence of CRS.

11. INFECTION PREVENTION

11.1. Prolonged B cell aplasia may occur after CAR-T therapy due to targeting normal B cells. This often results in hypogammaglobulinemia and increased risk of infection. The following preventive measures will be followed.

11.2. Anti-microbial prophylaxis

11.2.1. **Antiviral prophylaxis** (e.g. acyclovir 800mg BID) will start with lymphodepletion (unless patient is already on it) and continued for at least 12 months post therapy.

11.2.2. **Anti-PJP prophylaxis** (e.g. Bactrim DS BID twice weekly) will start at day +30 or upon stable count recovery (ANC >1,000 and platelet count > 50,000) for at least 6 months. If delayed count recovery (>4 weeks post CAR-T therapy), alternative agent (atovaquone or dapsone) will be used.

11.3. Anti-fungal prophylaxis will be used when:

11.3.1. ANC < 500/mm^3 and until stable ANC recovery >500 (fluconazole or caspofungin).

11.3.2. High-dose steroid for treatment of CRS or neurotoxicity (posaconazole or voriconazole as anti-mold prophylaxis).

11.4. Anti-bacterial prophylaxis: to start when ANC<500/mm³ until ANC is >500/mm³.

11.5. IVIG replacement (400 mg/kg/dose) is ONLY indicated with recurrent or prolonged infection and low IgG (<400 mg/dl), and it may be considered if IgG <200 mg/dl in vulnerable populations (elderly or with comorbidities). Thus, routine IgG monitoring may not be indicated in all patients. IgG level surveillance is continued until 4 weeks post infusion or longer if continues to be low with indication for replacement. If prolonged hypogammaglobulinemia (>6 months post infusion), long-term IgG replacement will be considered.

11.6. Vaccinations: There is no data to support routine vaccination post-CAR-T cells. Preclinical data showed persistence of long-lived plasma cells.

11.6.1. **Post-transplant vaccination** (i.e. in patients who had prior autologous or allogeneic hematopoietic cell transplant) or **pneumonia vaccine** (if applicable) are to be given starting 3 months post CAR-T cell infusion.

11.6.2. **Annual influenza vaccine** will be given during the corresponding season.

APPENDICES

11.7. APPENDIX A: OSUWMC Department of Pharmacy Tocilizumab Rounding

11.8. APPENDIX B: Definition of high-dose vasopressors

INTERNAL REFERENCES

- BMT 19 Infection Prophylaxis and Management
- Acute Leukemia Plan of Care 10 - Management of Tumor Lysis Syndrome

AUTHOR(S):

Samantha Jaglowski, Ayman Saad, Zeinab El Boghdadly, Karen Bishop Hill, Julianna Roddy, Emily Watson, Samantha Knight, Misty Lamprecht

INTERNAL REFERENCE(S): Tocilizumab Orderset.

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CHANGE CONTROL:

Section	Significant Change	Rationale
5.4.	**NEW:** Antiviral Prophylaxis: • For patients who are HBV or HCV positive, management will follow BMT 19 Infection Prophylaxis and Management of HCT. Referral to Hepatology is recommended for patients with active disease. Suppressive therapy for HBV (e.g. entecavir) is recommended (before starting lymphodepletion) for patients who are HBV positive.	Updated to current practice and NCCN Guidelines.

Prepared/Revised By: _____ **Date:** _____
Alison Neal, Senior Quality Manager

Reviewed/Approved By: _____ **Date:** _____
Samantha Jaglowski, MD, MPH BMT Clinical Director

Reviewed/Approved By: _____ **Date:** _____
Ayman Saad, MD, Professor of Hematology

Removed From Service Date: _____ **Initials:** _____

APPENDIX A: OSUWMC Department of Pharmacy Tocilizumab Rounding

- Per the Medication Rounding Policy, tocilizumab doses will be rounded to the nearest vial size. Please use the table below to identify the correct rounded dose per patient weight and number of vials to use when preparing this order.

Patient Weight (kg)	Rounded Dose (mg)	Number of Vials (80 mg)	Number of Vials (200 mg)	Number of Vials (400 mg)
50 - 54	400 mg	-	-	1
55 - 59	440 mg	3	1	-
60 - 64	480 mg	1	-	1
65 - 69	520 mg	4	1	-
70 - 74	560 mg	2	-	1
75 - 79	600 mg	-	1	1
80 - 84	640 mg	3	-	1
85 - 89	680 mg	1	1	1
90 - 94	720 mg	4	-	1
95 -99	760 mg	2	1	1
100 and above	800 mg	-	-	2

APPENDIX B: Definition of high-dose vasopressors

- Patient is rapidly declining over 2-3 hours with increasing vasopressor dose
- Patient remains on high dose vasopressor ≥ 3hours

Preferred Vasopressors	High Dose	Max
Norepinephrine monotherapy	≥0.5 mcg/kg/min	Usual: 1 mcg/kg/min
Phenylephrine monotherapy	>3mcg/kg/min (≥200 mcg/min)	9 mcg/kg/min
Alternative Vasopressors	**High Dose**	**Max**
Epinephrine monotherapy	≥0.5 mg/kg/min	Max 1 mcg/kg/min
Vasopressin + norepinephrine	≥0.3 mcg/kg/min (≥20mcg/min using VASST formula, assuming 70kg)	N/A
Dopamine monotherapy*	≥10 mcg/kg/min	20 mcg/kg/min

*Consider only as a 3rd or 4th line vasopressor

16.5 APPENDIX 5. LONG TERM FOLLOW UP CHECKLIST. YEARS 1 – 5 AFTER CAR-T CELL TREATMENT

CHECKLIST FOR CLINICAL VISIT FOR LONG TERM FOLLOW UP OF CLINICAL TRIAL OSU 21170_. YEARS 1 – 5 AFTER CAR-T CELL THERAPY FOR B CELL MALIGNANCIES	
DOCUMENT DATE AND TIME	▪ Document date and time ▪ Document time since anti-CD19 CAR-T cell therapy
HISTORY	▪ Perform clinical history ▪ Document new conditions ▪ New malignancy(ies) ▪ New incidence or exacerbation of a pre-existing neurologic disorder ▪ New incidence or exacerbation of a pre-existing rheumatologic or autoimmune disorder ▪ New incidence of a hematologic disorder.
EXPOSURE HISTORY	▪ Exposure to mutagenic agents ▪ Exposure to new medications
PHYSICAL EXAMINATION	▪ Perform physical examination
LABORATORY RESULTS	▪ CBC and differential ▪ Vector persistence assay
UPDATE STUDY CONTACT INFORMATION	▪ Give subject the latest contact information for the clinical trial coordinator and principal investigator.

16.6 APPENDIX 6. LONG TERM FOLLOW UP CHECKLIST. YEARS 6 – 15 AFTER CAR-T CELL TREATMENT

CHECKLIST FOR QUERY FOR LONG TERM FOLLOW UP OF CLINICAL TRIAL OSU 21170 . YEARS 6 - 15 AFTER CAR-T CELL THERAPY FOR B CELL MALIGNANCIES	
Subjects may be contacted during an in office clinical visit, via telephone or written questionnaire. If vector persistence testing needed, this will be obtained at the time of an office visit.	
DOCUMENT DATE AND TIME	▪ Document date and time ▪ Document time since anti-CD19 CAR-T cell therapy

HISTORY	▪ Perform clinical history
	▪ Document new conditions
	  ▪ New malignancy(ies)
	  ▪ New incidence or exacerbation of a pre-existing neurologic disorder
	  ▪ New incidence or exacerbation of a pre-existing rheumatologic or autoimmune disorder
	  ▪ New incidence of a hematologic disorder.

LABORATORY RESULTS	▪ Vector persistence assay (if previously positive)
UPDATE STUDY CONTACT INFORMATION	▪ Give subject the latest contact information for the clinical trial coordinator and principal investigator.

16.7 APPENDIX 7. PEDIATRIC CONSIDERATIONS

Pediatric Lymphodepletive Chemotherapy Regimen:

- Fludarabine 30 mg/m^2/day IV x 4 days (days -6 to -3)
- Cyclophosphamide 500 mg/m^2/day IV x 2 days starting with the first dose of Fludarabine (day -6 to -5)

Day -6	Fludarabine 30 mg/m^2	Cyclophosphamide 500 mg/m^2
Day -5	Fludarabine 30 mg/m^2	Cyclophosphamide 500 mg/m^2
Day -4	Fludarabine 30 mg/m^2	
Day -3	Fludarabine 30 mg/m^2	
Day -2 to -1	Rest	
Day 0	Anti-CD19/20/22 CAR T Cell infusion	
Day 7	Anti-CD19/20/22 CAR T Cell infusion	

Pediatric Cell Infusion Guidelines:

- Anti-CD19/20/22 CAR T cells will be infused per institutional guidelines for administration of fresh or frozen cell therapy products.
- The patient will receive pre-medication with acetaminophen (10-15 mg/kg, max 650 mg PO) and diphenhydramine (1 mg/kg PO or IV, max 50 mg) administered 30-60 minutes prior to cell infusion.
- Emergency medications should be available at the patient's bedside including: Diphenhydramine, epinephrine 1 mg/ml (0.01 mg/kg IM, max 0.5 mg) or appropriately dosed EpiPen.
- **It is recommended that patients not receive systemic corticosteroids such as hydrocortisone, prednisone, prednisolone (Solu-Medrol) or dexamethasone (Decadron) at any time, except in the case of a life-threatening emergency, since this may have an adverse effect on T cells.** If corticosteroids are required for an acute, life-threatening infusional reaction, an initial dose of hydrocortisone 2 mg/kg (max 100 mg) is recommended.
- A dose of tocilizumab must be confirmed as reserved and available in the pharmacy prior to infusion.
- Infusion should start at least 24h after the last dose of lymphodepleting chemotherapy.