

Stanford Cancer Institute

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Comprehensive Cancer Center



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TITLE:

Phase 1 Trial Evaluating the Safety of Myeloablative Conditioning, Orca-T,
and Allogeneic, Donor-Derived CD19/CD22-CAR (Chimeric Antigen
Receptor) T cells in Adults with B-cell Acute Lymphoblastic Leukemia (ALL)

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Single-Center

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Regulatory Information

Study Agent: Allogeneic donor-derived T-cells transduced with bivalent lentiviral vector (CD19/CD22-BBz); chimeric antigen receptor (CAR); Orca-T; intravenously

IRB of Record: Stanford Research Compliance Office

IND / IDE Sponsor: Melody Smith, MD, MS

IND / IDE Cross-reference: IND 17484 (Sponsor: Crystal Mackall); IND 018873 (Sponsor: Orca Bio)

Funding Source /:

PROTOCOL HISTORY

Amendment Date *	Change Summary
0 11 May 2022	Initial submission to SCI Scientific Review Committee (SRC)
1 14 June 2022	• Clarification of Recipient SOA procedure and footnotes, eligibility criteria • Revision to the CMV/EBV monitoring frequency based on prior research protocol implementation practices • Revision to the tacrolimus level from 6 to a range of 5 to 7 • Revised Section 6.3 - Procedures and Assessments Risk Information to reflect additional risk information • Clarification of DTL language per SRC pre-review comments • Administrative edits – formatting, listing of sub-investigators, study personnel, additions to LOA
2.0 02 August 2022	• Addition of sub-investigators • Addition of information for aGVHD • Clarification of DTL definition per SRC review comments • Addition of language to align with the required FDA language for retroviral vectors ○ Section 6.3.3 ○ Recipient informed consent form – Purpose of Research, Risk sections • Clarification of donor eligibility criteria • Addition of SRC approval information • Addition of revisions per FDA initial review ○ Addition of the IND number ○ Changes to the conditioning regimen ▪ Protocol schema updated ▪ Section 5.3.2 and 6.3.2 updated ○ Evaluation of engraftment revised from Day 60 to Day 42 ▪ Revision to the Recipient SOA to reflect addition of Day 42 • Recipient Consent Form - revised Recipient SOA ▪ Updated Synopsis, Section 5.8, Section 6.2.1, Section 7.5.2, Section 10.2.1, Section 10.3.2 ▪ Addition of Section 5.6.1 ○ Clarification of safety stopping rules, DLT definitions, and enrollment stagger plan ○ Clarification for collection of back-up graft in the event of graft failure events • Addition of language in Section 6.3.3.1.2 for antimicrobial prophylaxis language

Amendment Date *	Change Summary
3.0 23 November 2022	• Addition of sub-investigator and correction to sub-investigator name • Clarifications for the correlative table and section • Revision of table titles and numbers to improve protocol readability • Clarification throughout the protocol per Orca Bio, Inc. pertaining to Orca-T investigational product: ○ T_{con} added throughout to articulate, Orca-T is three phenotypically-distinct cellular products: conventional T cells (T_{con}), regulatory T cells (T_{reg}) and CD34+HSPCs (HSPC) to make up Orca-T ○ Revision of Section 4.2 to align with current Orca Bio, Inc. IND documentation • Revision of the vital signs monitoring timepoints • Clarification of the timeframe between the infusion of T_{con} and CD19/CD22-CAR T cells • Removal of Brain MRI from Day 1 • Removal of daily coagulation from Day 1 to Day 21 timeframe ○ Revised to twice weekly coagulation from Day 1 to Day 21 • Clarification of RCL sample on Day 1 (any time prior to infusion is appropriate for baseline) • Clarification of negative pregnancy test being prior to the initiation of chemotherapy regimen • Clarification of inclusion criteria for B-cell ALL definition ○ Inclusion of NCCN Guidelines Version 1.2022, Acute Lymphoblastic Leukemia as Appendix I • Clarification of lumbar puncture procedures • Revision of GVHD assessment ○ Revised to weekly rather than daily for Day 1 to Day 21 timeframe • Further clarification of DLT language per FDA recommendations from initial review • Revision of the donor collection schedule to allow flexibility for manufacturing operations ○ Clarification of two day mobilized and unmobilized collection timepoints and G-CSF doses • Clarification of AE collection and reporting language • Update of risk language to include risk of infection language included in the consent form • Clarification of tacrolimus administration to be according to institutional guidelines • Update to the Supportive Care/Continuation of Inpatient Monitoring period to include the consideration of outpatient monitoring per investigator discretion • Revision of RCL PCR testing operations in Section 6.2 and Appendix F • Removal of the following assessments, as they are not required for subject follow-up) during the Every 6 months, 1-5 years (-/+ 2 months): Physical Exam, Vital Signs, and Neurological Exam • Revision of protocol graphics to illustrate the current timepoints and operations of the study

Amendment Date *	Change Summary
3.1 29 March 2023	• Addition of NCT# to title page • Primary graft failure (Section 6.2.1) Revise WBC to ANC • Removal of Day +60 LP from Schedule of Assessments • Removal of 2nd MRD+ requirement for Recipient Inclusion Criteria and overall clarification of criteria • Revision of the vital signs language for clarification and consistency (Section 6.2.1) • Revision and clarification of primary graft failure (Section 6.2.1)
4.0 31 May 2023	• Addition of match unrelated donors to Donor Inclusion Criteria and throughout sections of the protocol • Addition of National Marrow Donor Program (NMDP) to List of Abbreviations & Terms and Section 5.1 • Minor administrative revisions to streamline protocol text
4.1 06 September 2023	• Addition of minor donors (≥ 16) to donor eligibility criteria

5.0 15 January 2025	• Addition of language that states use of NMDP documentation to confirm donor eligibility • Revision and clarification of the dose stagger cohort with the following language: ◦ Once the dose escalation cohorts are complete with no DLTs, that the dose expansion portion of the protocol does not require a stagger. • Clarification of tacrolimus language ◦ Clarification for study calendar (tacrolimus starts on Day 3) ◦ Instead of referencing institutional policy, revised to reference the protocol ▪ Tacrolimus starts the day after T_{con} infusion, but not sooner than 12 hours • Clarification that recipient/donor with CMV negative statuses <u>do not require</u> CMV testing • Clarification of CAR-T infusion per institutional guidelines • Clarification of Keppra being administered per institutional guidelines • Clarification that dose adjustments made per institutional guidelines: ◦ Clarification of cyclophosphamide administered per institutional guidelines • Additional non-conforming language for investigational products, Section 6.3.5: ◦ <u>If a product(s) does not meet the requirements for subject infusion, for whatever reason (manufacturing failure, out-of-specification, lost or damaged product in transit, bag failure, etc), the subject will be monitored, and the investigators will determine the most appropriate treatment plan.</u> <u>Specifically, if a product is out-of-specification (OOS), the Sponsor-Investigator will decide if the subject will be.</u> • Language for non-conforming products added to the recipient consent form • Section 6.2.2, additional language for T and B cell lymphocyte subsets • Addition of language for initial and prolongation of hospitalization to SAE definition reporting criteria ◦ Additional language for NCI definition for hospital stays defined as 24 hours or longer • Clarification of donor AE language - only collecting AEs that are unexpected in the EDC • Clarification of AESIs language ◦ Section 7.2.4: • Grade 2 or higher infections <u>(within the first year following Orca-T and CAR-T)</u> ◦ <u>With the exception of thrush, UTI, uncompleted respiratory viruses, minor skin infections</u> ◦ Section 7.2.5: ▪ <u>New incidence of infections that occur beyond one year will be collected only if they are</u> potentially related to investigational CD19/CD22-CAR T cells • Clarification and revision of recipient adverse event collection ◦ Section 7.5.2: • <u>All non-hematologic laboratory AEs will not be collected with the exception of those deemed clinically significant.</u> • Addition of Section 8.2.1.1 to include language outlining specific post-treatment sample collection ◦ Language added to the consent form
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Amendment Date *	Change Summary
	• Section 8.2.3 and Section 8.2.3.7, addition and expansion of intestinal microbiome (including stool) language for correlative research ○ Language added to the consent form • Updates to the correlative table, 12.6 Appendix E ○ Footnote 11 added to Flow panel, Day 7 and Day 14 timepoints ○ Footnote 12 added to CAR qRT-PCR, Day 7 and Day 14 timepoints ○ Intestinal microbiome analysis, Day 10 and Day 21 timepoints removed • In Section 12.7, removal of following language to reflect current operations: ○ Archive samples annually thereafter for an additional 4 years ($\pm$ 2 months), if previous samples have been negative. <u>SOA Revisions:</u> • Removal coagulation studies (Day 2) • Addition of +/- 4 day window for Day 42 timepoint • Revision of MRD blood sample, made optional at all timepoints • Removal of "Weekly from Day 7 to Day 60" subtext for EBV, CMV lab • Addition of T&B (T and B cell lymphocyte) subsets (SOA): ○ Day 30, Day 60, Months 3, 6, 12, then yearly

* Latest version date should match footer date of the current protocol

STATEMENT OF COMPLIANCE

SPONSOR-INVESTIGATOR STATEMENT

I have read and agree to the study, as detailed by this protocol document. I am aware of my responsibilities as an Investigator pursuant to the clinical trial protocol, the guidelines of [Good Clinical Practice \(GCP\)](#)¹, the Declaration of Helsinki², and the applicable Code of Federal Regulations (CFR) at [Title 21](#)³ and [Title 45 Part 46](#)⁴, as well as my responsibilities as a Sponsor-Investigator and IND/IDE-holder under 21 CFR §312 or §812³, as a ClinicalTrials.gov Responsible Party under [42 CFR Part 11](#)⁵ as promulgated pursuant to Section 801 of the Food and Drug Administration Amendments Act (FDAAA 801), and other applicable requirements of the participating institutions including the Stanford Cancer Institute, the Stanford Hospitals and Clinics, Lucile Packard Children's Hospital, and/or the Stanford University Medical Center. I agree to conduct the trial according to these regulations, guidelines, and requirements, and to appropriately direct and assist the participating staff under my authority, and that all staff members are aware of, and trained in, their clinical trial responsibilities.

All key study personnel have completed Human Subjects Protection Training.

Site Principal Investigators are expected to assure that no deviation from, or changes to the protocol, will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB) of record, except where necessary to eliminate an immediate hazard(s) to the trial subjects.

Sponsor-Investigator's Name:

Name of Site: Stanford Cancer Institute
Stanford Medicine
Stanford, California

Principal Investigator's Signature: _____ **Date:** _____

- 1 FDA Guidance for Industry: current revision of the International Conference on Harmonization (ICH) Good Clinical Practice Guidelines E6.
- 2 World Medical Association, Declaration of Helsinki Ethical Principles for Medical Research Involving Human Patients.
- 3 United States Code of Federal Regulations (CFR), [Title 21, "Food and Drugs."](#)
- 4 CFR, [Title 45 "Public Welfare;" Part 46 "Protection of Human Subjects."](#)
- 5 CFR, [Title 42 "Public Health;" Part 11 "Clinical Trials Registration and Results Information Submission."](#)

STATEMENT OF COMPLIANCE

PARTICIPATING SITE PRINCIPAL INVESTIGATOR STATEMENT

I have read and agree to the study, as detailed by this protocol document. I am aware of my responsibilities as an Investigator pursuant to the clinical trial protocol, the guidelines of [Good Clinical Practice \(GCP\)](#)¹, the Declaration of Helsinki², and the applicable Code of Federal Regulations (CFR) at [Title 21](#)³ and [Title 45 Part 46](#)⁴, and the applicable requirements of the participating institution. I agree to conduct the trial according to these regulations, guidelines, and requirements, and to appropriately direct and assist the participating staff under my authority, and that all staff members are aware of, and trained in, their clinical trial responsibilities.

All key study personnel have completed Human Subjects Protection Training.

I assure that no deviation from, or changes to the protocol, will take place without prior agreement from the sponsor and documented approval from the Institutional Review Board (IRB) of record, except where necessary to eliminate an immediate hazard(s) to the trial subjects.

Principal Investigator's Name: Lori Muffly, MD, MS

Name & address of local site:

Name of Lead Institution: Stanford Cancer Institute
Stanford Medicine
Stanford, California

Principal Investigator's Signature: _____ **Date:** _____

- 1 FDA Guidance for Industry: current revision of the International Conference on Harmonization (ICH) Good Clinical Practice Guidelines E6.
- 2 World Medical Association, Declaration of Helsinki Ethical Principles for Medical Research Involving Human Patients.
- 3 United States Code of Federal Regulations (CFR), [Title 21, "Food and Drugs."](#)
- 4 CFR, [Title 45 "Public Welfare;" Part 46 "Protection of Human Subjects."](#)

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NCCN Guidelines Version 1.2022 Acute Lymphoblastic Leukemia

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CYTOGENETIC RISK GROUPS FOR B-ALL

RISK GROUPS	CYTOGENETICS
Good risk	- Hyperdiploidy (51–65 chromosomes) ▶ Cases with trisomy of chromosomes 4, 10, and 17 appear to have the most favorable outcome - t(12;21)(p13;q22): <i>ETV6-RUNX1</i>^a
Poor risk	- Hypodiploidy^{b,c} (<44 chromosomes) <i>KMT2A</i> rearranged (t[4;11] or others) - t(v;14q32)/IgH - t(9;22)(q34;q11.2): <i>BCR-ABL1</i> (defined as high risk in the pre-TKI era) - Complex karyotype (5 or more chromosomal abnormalities) <i>BCR-ABL1</i>-like (Ph-like) ALL ▶ JAK-STAT (<i>CRLF2</i>;^{c,d} <i>EPOR</i>, <i>JAK1/2/3</i>, <i>TYK2</i>, mutations of <i>SH2B3</i>, <i>IL7R</i>, <i>JAK1/2/3</i>) ▶ ABL class (rearrangements of <i>ABL1</i>, <i>ABL2</i>, <i>PDGFRA</i>, <i>PDGFRB</i>, <i>FGFR</i>) ▶ Other (<i>NTRK</i>, <i>FLT3</i>, <i>LYN</i>, <i>PTK2B</i>) - Intrachromosomal amplification of chromosome 21 (iAMP21) - t(17;19): <i>TCF3-HLF</i> fusion - Alterations of <i>IKZF1</i>^{e,f}

^a The translocation t(12;21)(p13;q22) is typically cryptic by karyotyping and requires FISH or PCR to identify.

^b There are other results that are not less than 44 chromosomes that may be equivalent to hypodiploidy and have the same implications. It is important to distinguish true hypodiploidy from masked hypodiploidy, which results from the doubling of hypodiploid clones. Carroll AJ, Shago M, Mikhail FM, et al. Masked hypodiploidy: Hypodiploid acute lymphoblastic leukemia (ALL) mimicking hyperdiploid ALL in children: A report from the Children's Oncology Group. *Cancer Genet* 2019;238:62-68.

^c Alternatively defined as DNA index less than protocol-defined threshold or other clear evidence of hypodiploid clone. Hypodiploid ALL is also often associated with *TP53* loss of function mutations and Li-Fraumeni syndrome.

^d Jain N, Roberts KG, Jabbour E, et al. Ph-like acute lymphoblastic leukemia: a high-risk subtype in adults. *Blood* 2017;129:572-581; Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activation lesions in Ph-like acute lymphoblastic leukemia. *N Engl J Med* 2014;371:1005-1015.

^e Mullighan CG, Su X, Zhang J, et al. Deletion of *IKZF1* and prognosis in acute lymphoblastic leukemia. *N Engl J Med* 2009;360:470-480; Stanulla M, Dagdan E, Zaliouva M, et al. *IKZF1*plus defines a new minimal residual disease-dependent very-poor prognostic profile in pediatric B-cell precursor acute lymphoblastic leukemia. *J Clin Oncol* 2018;36:1240-1249.

^f Emerging evidence suggests *DUX4* ALL is favorable. Additionally in cases of *DUX4*, *IKZF1* alterations do not confer poor prognosis.

Note: All recommendations are category 2A unless otherwise indicated.

Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

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ALL-A
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PROTOCOL SYNOPSIS

Study Title	Phase 1 Trial Evaluating the Safety of Myeloablative Conditioning, Orca-T, and Allogeneic, Donor-Derived CD19/CD22-CAR (Chimeric Antigen Receptor) T cells in Adults with B-cell Acute Lymphoblastic Leukemia (ALL)
Study Drug, Device, or Procedure (select)	Procedure
Study Description	Open-label, dose-escalation, single group, non-randomized, non-controlled single center
Study Phase	Phase 1
Study Purpose (for ClinicalTrials.gov)	Treatment
Primary Objectives	1. Assess the feasibility of manufacturing and the safety of administering allogeneic, donor CD19/CD22-CAR T cells that meet established release specifications and to determine the MTD in adults with B-cell acute lymphoblastic leukemia (ALL) following a myeloablative conditioning regimen and the Orca-T graft (purified regulatory T-cells [T_{reg}], CD34+ hematopoietic progenitor cells [HSPC], and conventional T-cells [T_{con}]).

Hypothesis

For patients with ALL receiving a myeloablative conditioning regimen and the Orca-T graft from a matched-related or unrelated donor, the infusion of donor conventional CD19/CD22-CAR T cells will augment graft versus leukemia without increasing acute graft versus host disease (GVHD) or graft failure.

Primary Endpoints

1. Ability to manufacture donor CD19/CD22-CAR T cells that meet established release specifications will be assessed by the number of product successes – with a goal of 2 of 3 products, 3 of 6 products, 4 of 12 products, or 5 of 18 products.
2. Safety of the donor CD19/CD22-CAR T cells plus the Orca-T graft will be assessed by the incidence of engraftment without Grade III to IV acute GVHD at Day 42 to determine the MTD.

Secondary Objectives

1. Determine the cumulative incidence of relapse or progression of disease as well as non-relapse mortality (NRM) following donor CD19/CD22-CAR T cells plus Orca-T infusion.
2. Assess the rate of secondary graft failure following donor CD19/CD22-CAR T cells plus Orca-T infusion.
3. Analyze progression-free (PFS) and overall (OS) survival following a donor CD19/CD22-CAR T cell infusion plus Orca-T infusion.
4. Evaluate the incidence of greater than Grade 3 infectious disease complications

Secondary Endpoints

1. Relapse, disease progression and NRM at 1 year
2. Frequency of secondary graft failure within 1 year
3. PFS and OS at 1 year following Orca-T infusion plus donor CD19/CD22-CAR T cell infusion
4. Greater than Grade 3 infectious disease complication within 1 year

Exploratory Analyses

1. Measure persistence of T_{cons}, T_{regs}, and donor CD19/CD22-CAR T cells in the blood, bone marrow and CSF
2. Investigate donor CD19/CD22CAR T cell properties correlated with CAR T cell efficacy and persistence
3. Evaluate the impact of donor CD19/CD22-CAR T cells on immune reconstitution after Orca-T infusion including its impact on numbers of circulating B cells and bone marrow
4. Assess the impact of the donor CD19/CD22-CAR T cells on the genomic profile of the patients with CAPP-seq compared to historic controls
5. Investigate cytokine signature of the donor CD19/CD22-CAR T cells as compared to CD19/CD22-CAR T cells from autologous sources
6. Assess the impact of the donor CD19/CD22-CAR T cells on the ALL-stem cell and the bone marrow microenvironment
7. Evaluate whether donor CD19/CD22-CAR T cells traffic to GVHD target organs

Indication

Patients with high-risk B-cell ALL who are candidates for allogeneic hematopoietic cell transplantation will be eligible for enrollment on the study. The patients will have an HLA matched related or unrelated donor identified.

Sample Size

The study will consist of 12-18 patients in three cohorts consisting of 3 to 9 patients per cohort

Eligibility Criteria**Patient Inclusion Criteria**

- Disease Status of B-cell ALL
 - Subjects in CR must have a history of chemotherapy refractory disease defined as progression OR stable disease after one line of chemotherapy, OR relapsed disease after achieving prior CR, OR a history of residual disease in the peripheral blood or bone marrow (by flow cytometry, PCR, FISH, or next generation sequencing) following induction or consolidation therapy OR must have other high risk ALL features per the NCCN Guidelines (Version 1.2022), Acute Lymphoblastic Leukemia (Appendix I)
 - Subjects with active ALL (defined as $\geq 5\%$ bone marrow blasts, circulating blasts, or extramedullary disease) are eligible.
- Age ≥ 18 and ≤ 65 years (i.e., from age 18 to < 66 years old) at the time of enrollment
- Eastern cooperative oncology group (ECOG) performance status of 0, 1, or 2; or Karnofsky $\geq 60\%$
- CD19 expression is required any time since diagnosis. CD19 expression may be detected by immunohistochemistry or by flow cytometry. The choice of whether to use flow cytometry or immunohistochemistry will be determined by what is the most easily available tissue sample in each subject. In general, immunohistochemistry will be used for lymph node biopsies, flow cytometry will be used for peripheral blood and bone marrow samples. Patients receiving prior CD19 CAR T cell or blinatumomab are eligible if there is no documented history of CD19 negativity on the malignant cells.
- Subjects must have an HLA matched related or unrelated donor willing to undergo unstimulated apheresis for T cell collection for CAR T cell generation followed by GCSF mobilized apheresis for HSC/Treg graft.

- Matched related or unrelated donor who is an 8/8 match for HLA-A, -B, -C, and -DRB1, all typed using DNA-based high-resolution methods
- Subjects must have adequate organ function measured by:
 - Cardiac: Cardiac ejection fraction at rest $\geq 45\%$
 - Hepatic:
 - Total bilirubin < 2 times upper limit of normal (ULN) (patients with Gilbert's syndrome may be included where hemolysis has been excluded and with approval of the study PI)
 - ALT/AST ≤ 3 times ULN
 - Renal: Calculated creatinine clearance ≥ 50 mL/min or serum creatinine < 2.0 mg/dL
 - Pulmonary: Diffusing capacity of the lung for carbon monoxide (DLCO) (adjusted for hemoglobin) $\geq 50\%$
 - CNS: Subjects with CNS involvement are eligible as long as there are no overt signs or symptoms that in the evaluation of the investigator would mask or interfere with the neurological assessment of toxicity.
- Negative serum or urine beta-HCG test in females of childbearing potential within 3 weeks of registration
- Subjects of childbearing or child fathering potential must be willing to practice birth control from the time of enrollment on this study and for twelve (12) months after receiving the preparative conditioning regimen.
- Must be able to give informed consent. Legal authorized representative (LAR) is permitted if subject is cognitively able to provide verbal assent.

Patient Exclusion Criteria

- History of other malignancy unless disease free for at least 3 years. At the discretion of the Principal Investigator, subjects in remission for 1-2 years prior to enrollment may be deemed eligible after

considering the nature of other malignancy, likelihood of recurrence for one year following therapy, and impact of prior treatment on risk of CD19/CD22-CAR T cells and Treg graft. Subjects in remission <1 year are not eligible. The following exceptions apply:

- Nonmelanoma skin cancer or carcinoma in situ (e.g. cervix, bladder, breast) is eligible.
 - Hormonal therapy in subjects in remission >1 year will be allowed.
- Patients who have undergone a prior allogeneic or autologous stem cell transplant.
- Recipient positive anti-donor HLA antibodies against a mismatched allele in the selected donor determined by either:
 - a positive crossmatch test of any titer (by complement-dependent cytotoxicity or flow cytometric testing), or
 - the presence of anti-donor HLA antibody to any of the following HLA loci: HLA-A, -B, -C, -DRB1, -DQB1, -DQA1, -DPB1, or -DPA1, with mean fluorescence intensity (MFI) >1000 by solid phase immunoassay
- Presence of fungal, bacterial, viral, or other infection that is uncontrolled. Simple UTI and uncomplicated bacterial pharyngitis are permitted if responding to active treatment.
- Known history of infection with any of the following:
 - HIV
 - Hepatitis B (HBsAg positive) **
 - Hepatitis C virus (anti HCV positive) **

** A history of hepatitis B or hepatitis C is permitted if the viral load is undetectable per quantitative PCR and/or nucleic acid testing.

- Currently receiving corticosteroids or other immunosuppressive therapy. Topical corticosteroids or oral systemic corticosteroid doses less than or equal to 10 mg/day are allowed.
- Planned pharmaceutical in vivo or ex vivo T cell depletion, e.g., post-transplant cyclophosphamide (Cy), peri-transplant anti-

thymocyte globulin (ATG), or alemtuzumab. For patients that have previously been exposed to a T cell-depleting agent, a 5-half-life washout of the agent must occur prior to planned Transplant Day 0.

- Hyperleukocytosis ($\geq 50,000$ blasts/ μL) or rapidly progressive disease that in the estimation of the investigator and sponsor would compromise ability to complete study therapy.
- History of myocardial infarction, cardiac angioplasty or stenting, unstable angina, or other clinically significant cardiac disease within 12 months of enrollment.
- Pregnant or breast feeding
- Patients with known autoimmune disease requiring the use of systemic immunosuppressive therapy within the last year
- Presence of a seizure disorder, cerebrovascular ischemia/hemorrhage, dementia, cerebellar disease, or any autoimmune disease with CNS involvement that in the judgment of the investigator may impair the ability to evaluate neurotoxicity.
- Any medical condition that in the judgement of the investigator is likely to interfere with assessment of safety or efficacy of study treatment

Donor Inclusion Criteria

- Age ≥ 16 and ≤ 75 years at time of enrollment
- Karnofsky performance status of $\geq 70\%$ defined by institutional standards
- Willing to donate for two separate apheresis collections (T cells and PBSC)
- Matched related or unrelated donor who is an 8/8 match for HLA-A, -B, -C, and -DRB1, all typed using DNA-based high-resolution methods
- Negative serum or urine beta-HCG test in females of childbearing potential within 2 weeks of first apheresis
- Seronegative for HIV-1 RNA PCR; HIV 1 and HIV 2 Ab (antibody); HTLV-1 and HTLV-2 Ab; PCR negative or sAg (surface antigen)

negative for hepatitis B; negative for the *Treponema pallidum* antibody Syphilis screen; and negative for HIV-1 and hepatitis C by nucleic acid testing (NAT) within 40 days of donor apheresis procedures.

- In the case that *T. pallidum* antibody tests are positive, donors must:
 - Be evaluated and show no evidence of syphilis infection of any stage by physical exam and history
 - Have completed effective antibiotic therapy to treat syphilis
 - Have a documented negative non-treponemal test (such as RPR) or in the case of a positive non-treponemal test must be evaluated by an infectious disease expert to evaluate for alternative causes of test positivity and confirm no evidence of active syphilitic disease

Donor Exclusion Criteria

- Evidence of active infection
- Seropositive for HIV-1 or -2, HTLV-1 or -2
- Positive PCR test results indicating acute or chronic HBV infection. Patients with isolated HBV core antibody positivity will not be excluded. Donors whose HBV infection status cannot be determined conclusively by serologic test results (www.cdc.gov/hepatitis/hbv/pdfs/serologicchartv8.pdf) must be negative for HBV by PCR to be eligible for study participation.
- Potential for Zika virus infection as defined as any of the following:
 - Medical diagnosis of Zika virus infection in the past 6 months
 - Residence in, or travel to, an area with active Zika virus transmission within the past 6 months.
 - Unprotected sex within the past 6 months with a person who is known to have either of the risk factors listed above

Donors determined to be ineligible based on the results of Zika virus screening may be determined to be eligible if:

- The donor has no signs or symptoms consistent with active Zika virus infection and
- Either of the following is true:
 - The donor is a first-degree or second-degree blood relative of the recipient

- Urgent medical need, meaning no comparable human cell product is available and the recipient is likely to suffer death or serious morbidity without the human cell product, as attested by the Investigator.
- Pregnant or breastfeeding female
- Medical, physical, or psychological reason that would place the donor at increased risk for complications from growth factor or leukapheresis.

Dose

The doses below will be used for the donor derived CD19/CD22CAR T cells ($\pm 20\%$). We will employ a Bayesian dose escalation design.

Dose Level	CD3 ⁺ T _{con} Dose	CAR T cell Dose
-1	3x10 ⁶ cells/kg	0.5x10 ⁶ CAR+ cells/kg
1	3x10 ⁶ cells/kg	1x10 ⁶ CAR+ cells/kg
2	3x10 ⁶ cells/kg	2x10 ⁶ CAR+ cells/kg
3	3x10 ⁶ cells/kg	3x10 ⁶ CAR+ cells/kg

Treatment and Procedural Summary Following enrollment, the patient's allograft donor will have a leukapheresis product obtained from peripheral blood before stem cell mobilization to generate the CAR T cells. T cells will be enriched from the leukapheresis product, activated and genetically modified (using a lentiviral vector) to express a CAR targeting CD19/CD22 (19-22-BBz). These T cells will be expanded, and after the appropriate number of cells are generated, the modified T cells may be cryopreserved according to standard operating procedures for later infusion. A second G-CSF mobilized leukapheresis will be obtained for the allograft. The second leukapheresis will be the source of the following infusion products: conventional T-cells (T_{con}), regulatory T cells (T_{reg}), and CD34+ hematopoietic progenitor/stem cells (HSPC) (the three products make "Orca-T"). Patients will receive a myeloablative preparative regimen consisting of chemotherapy +/- total body irradiation. On Transplant Day 0, patients will receive the HSPC and T_{reg} infusions. On Day +2, patients will receive an infusion of conventional T cells (T_{con}) followed by infusion of donor CD19/CD22-CAR T cells. Graft-versus-host disease prophylaxis will initially commence on Day +3 with single agent tacrolimus (recommended target level 6). Serial sampling of blood and bone marrow will be performed following treatment to assess toxicity, therapeutic effects, and persistence of the genetically modified T cells.

**Statistical
Considerations**

- Feasibility will be defined as the ability to successfully manufacture donor CD19/CD22-CAR T cells at the target dose level. Feasibility will be assessed after 3, 6, 12, and 18 patients are treated. If feasibility is not met in 2 of 3 patients, 3 of 6 patients, 4 of 12, or 5 of 18 patients, the feasibility endpoint will not be met. Three (3) subjects may be replaced due to inability to achieve target dose of cells, and up to 3 subjects may be replaced for subjects enrolled who cannot receive cells for a maximum of 18 subjects.
- Stopping rules that will lead to a pause in the study will be assessed for patients within a given dose level:
 - *Episode of Grade 3 or 4 ICANS greater than 7 days:* Monitor the number of patients who experience this episode when either 3 or 6 patients have been treated at a dose level. Pause the study if the number is at least 2 out of 3 or at least 3 out of 6.
 - *Graft Failure by Day 42:* Monitor the number of patients who experience graft failure when either 3, 6, or 9 patients have been treated at a dose level. Pause the study if the number is at least 2 out of 3, at least 3 out of 6, or at least 4 out of 9.
 - Any death attributable to the product occurring at any dose level within 6-weeks of Day +2 (e.g., CAR T cell infusion) should trigger a pause in further enrollment until review by the appropriate Stanford committees
- CAR-T dose escalation: There are four dose levels of donor CD19/CD22-CAR T cells planned. The dose levels are as follows: 0.5×10^6 CAR+ cells/kg (Dose Level -1), 1×10^6 CAR+ cells/kg (Dose Level 1 – starting point), 2×10^6 CAR+ cells/kg (Dose Level 2), and 3×10^6 CAR+ cells/kg (Dose Level 3).

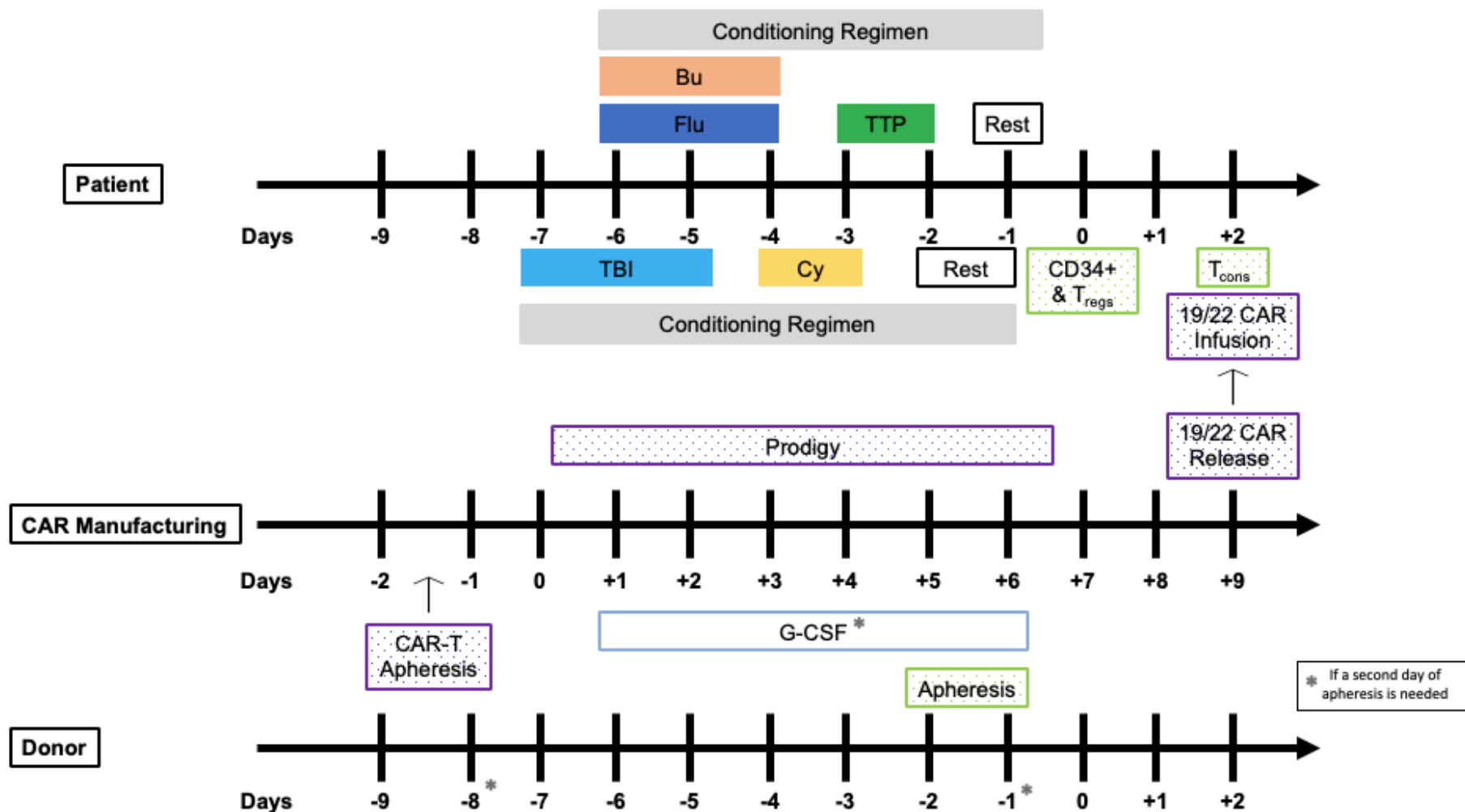
Study Duration

The recruitment period for this study is expected to be 2 years. Subjects will be followed for 15 years following treatment with CD19/CD22-CAR T cells. The total duration of this study is expected to be 1 to 2 years of active treatment and short term follow up. Subsequently, the patients will have a total of 15 years of long term follow up after the last subject completes study therapy due to ongoing monitoring required following CAR T cell therapy.

Subject Duration

Subject's active participation in this study is expected to be 2 years, with a total of 15 years of long term follow up from the time of cell infusion for the last patient.

PROTOCOL SCHEMA



LIST OF ABBREVIATIONS & TERMS

Accrual date	Subject's on-study date (per OnCore)	ICMJE	Int'l Committee of Med Journal Editors
AE	Adverse event	IV	Intravenous
AML	Acute myeloid leukemia	KPS	Karnofsky performance status
ALL	Acute Lymphoblastic Leukemia	IRB	Institutional Review Board
Allo-HCT	Allogeneic Hematopoietic Cell Transplant	LLN	Lower limit of normal
ALT	Aspartate aminotransferase	LVEF	Left ventricular ejection fraction
AST	Alanine aminotransferase	MDS	Myelodysplastic syndrome
BID	<i>bis in die</i> (twice-a-day)	MNC	Mononuclear cell
BSA	Body surface area	MRI	Magnetic resonance imaging
CBC	Complete blood count	MUGA	Multigated acquisition scan
		NAT	Nucleic acid testing
CFR	Code of Federal Regulations	NCI	National Cancer Institute
		NMDP	National Marrow Donor Program
CI	Confidence interval	NRM	Non-relapse mortality
C _{max}	Maximum concentration of drug	OnCore	OnCore Enterprise Research System
CMV	Cytomegalovirus	On-study date	Defined (per OnCore) as date of subject's first research-related procedure, scan, or treatment
CNS	Central nervous system	OS	Overall survival
CR	Complete response		
CRF	Case report / record form	PD	Progressive disease, tumor progression
CRi	Complete response with incomplete blood count recovery	PFS	Progression-free survival
CRp	Complete response with incomplete platelet recovery	PLT	Platelet
CTCAE	Common Terminology Criteria for Adverse Events	PR	Partial response
DLI	Donor leukocyte infusion	RECIST	Response evaluation criteria in solid tumors
DLCO	Diffusing capacity – lung for carbon monoxide	RR	Response rate
DLT	Dose-limiting toxicity	SAE	Serious adverse event
DSMB	Data Safety Monitoring Board	SCI	Stanford Cancer Institute
DSMC	Data Safety Monitoring Committee	SD	Stable Disease
DSMP	Data Safety Monitoring Plan	SGOT	Serum glutamic oxaloacetic transaminase
EBV	Epstein–Barr virus	SIN	Subject identification number
ECHO	Echocardiogram	SRC	Scientific Review Committee
ECOG	Eastern Cooperative Oncology Group		

FDA	Food and Drug Administration		
		Stanford IRB	Administrative Panels on Human Subjects in Medical Research
GCP	Good Clinical Practice	TBI	Total body irradiation
GRFS	GVHD-relapse-free survival	T _{con}	Conventional T cells (component of Orca-T)
GVHD	Graft Versus Host Disease	TLI	Total lymphoid irradiation
GVT	Graft-versus-tumor		
HCT	Hematopoietic cell transplant	T _{reg}	Regulatory T cells (component of Orca-T)
Hgb	Hemoglobin		
HIV	Human immunodeficiency virus	TTP	Time-to-progression
HPCA	Hematopoietic progenitor cell assay	ULN	Upper limit of normal
HSPC	Hematopoietic Stem and Progenitor Cells (component of Orca-T)	WBC	White blood cell
ICH	Int'l Conference on Harmonization		
ICANS	Immune Effector Cell-Associated Neurotoxicity Syndrome		
		WHO	World Health Organization

1 INTRODUCTION

1.1 Study Rationale

We hypothesize that for patients with ALL receiving myeloablative conditioning followed by HSPC, T_{reg} , and T_{con} infusions (Orca-T) from a matched-related or unrelated donor, the infusion of donor CD19/CD22-CAR T cells will augment graft versus leukemia without increasing acute graft versus host disease (GVHD) or graft failure.

The reasons for conducting the clinical trial are noted below:

- Administering donor CD19/CD22-CAR T cells after Orca-T infusion is a powerful strategy to enhance the graft-versus-tumor effect (GVT) following allogeneic hematopoietic cell transplant (allo-HCT).
- This approach will also provide the platform to assess whether the donor CD19/CD22-CAR T cells have increased efficacy with a decreased bulk of disease.
- Following myeloablative conditioning, the CAR-modified T cells should be able to expand given the lymphodeplete state of the recipient.
- The donor CD19/CD22-CAR T cells will be infused prior to the initiation of pharmacologic GVHD prophylaxis with tacrolimus, which will enable the CAR-modified T cells to expand prior to any potential blunting of their expansion by the immunosuppressant¹.

1.2 Background

1.2.1 Allogeneic hematopoietic cell transplantation for adults with B-ALL

Progress has been made in the therapeutic options for adults with B-cell ALL, yet allo-HCT remains an important component of curative intent therapy for patients with high-risk disease. A recent consensus criteria statement by experts in the field concluded that allo-HCT should be offered as consolidation therapy to adults with high-risk ALL (typically determined by cytogenetic/molecular risk group) in first complete remission (CR1) and to those in second or later complete remission (CR2+)². The authors conclude that allo-HCT may also be offered to patients with persistent measurable residual disease (MRD), as these patients are at high risk for disease progression with traditional chemotherapy consolidation. In the setting of refractory ALL, allo-HCT can be considered as a salvage option for patients who are refractory to other therapies, although this indication is more controversial.

The standard approach to allo-HCT for high-risk ALL in acceptable patients is with the use of a myeloablative conditioning regimen, typically including high dose chemotherapy with or without total body irradiation followed by infusion of an unmanipulated, human leukocyte antigen (HLA) compatible donor graft that includes CD34+ hematopoietic stem and precursor cells (HSPC) in addition to donor T-cells. With this standard approach, data from Stanford as well as published literature suggests that approximately 25% of patients will experience disease relapse following HCT, 15-20% will experience non-relapse mortality (NRM), typically related to graft-versus-host disease (GVHD) and 50-65% will become long-term survivors. Importantly, significant acute and chronic GVHD are detrimental side effects of HCT, leading to debilitation and reduced quality of life in survivors, prompting the development of a new endpoint in the field: GVHD-relapse-free

survival (GRFS). Our historical data, and that of others, suggests that GRFS rates in adults undergoing HCT for ALL is only approximately 25%.

1.2.2 T_{reg} , HSPC, and T_{con} (Orca-T) to reduce GVHD and improve NRM in myeloablative allogeneic HCT

To reduce GVHD associated with HCT, our group and others have focused on exploiting the protective effect of donor regulatory T cells (T_{regs}) in allo-HCT. T_{regs} are defined by high surface expression of CD4 and CD25, low-level expression of CD127 (IL-7R), and intracellular expression of the canonical transcription factor FoxP3³. Murine models and early clinical studies have suggested that enriching donor grafts with donor T_{regs} could improve allo-HCT by facilitating immune reconstitution and reducing the risk of GVHD while retaining GVT effects⁴⁻⁶.

Our group developed a graft engineering strategy using immunomagnetic selection and high-speed flow cytometric sorting to select CD4⁺CD25⁺ CD127^{lo} highly purified T_{regs} and conducted a Phase I/II study (NCT01660607) in which following myeloablative conditioning therapy, donor CD34⁺ HSPCs (HSPCs) were administered on the same day as purified T_{regs} , followed 2 to 3 days later by infusion of conventional donor T cells (T_{cons})⁷. In this protocol, fresh T_{regs} and T_{cons} were administered in a 1:1 ratio, and tacrolimus was administered as single agent GVHD prophylaxis.

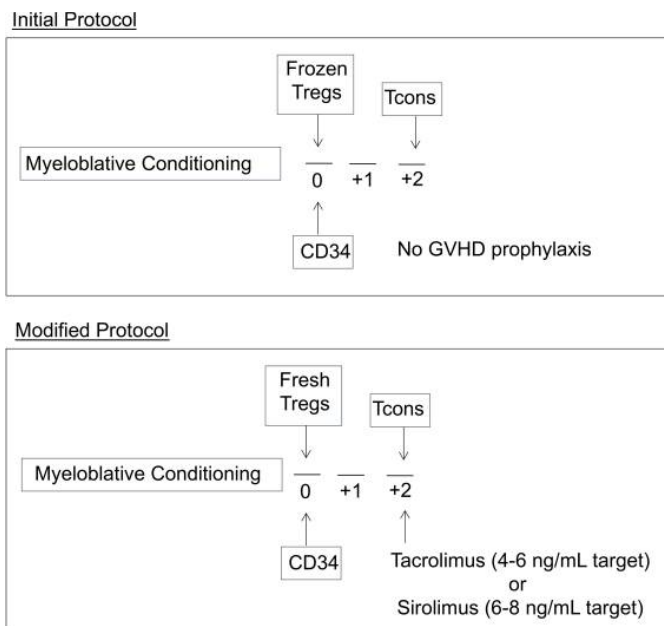


Figure 1. Protocol Schema for NCT01660607

In a report of 50 adult patients with hematologic malignancies treated using this T_{reg} CD34⁺ HSPC protocol, of which 14 patients had ALL, and 23% had active leukemia at time of HCT, no patients experienced NRM within the first year, and the GRFS at one year was approximately 75% (Meyer et al., ASH 2020). In an ongoing Phase Ib/II study (NCT04013685), 113 patients have been treated with this approach, now termed the Orca-T graft, and the 1-year GRFS was reported at 77% whereas the 1-year GRFS in a historical comparative cohort was 34% (Hoeg et al., ASH 2021). Although the Orca-T graft is highly promising and appears to reduce NRM and transplant-related toxicity, relapse occurs in over 20% of patients. Relapse after allo-HCT is associated with dismal outcomes for adults with ALL. Hence, further efforts aimed at reducing post-allo-HCT relapse with this allograft are needed.

1.2.3 Background on 19-22-BBz CAR T cells for patients with ALL

In recent years, cellular therapies have transformed the therapeutic landscape of relapsed/refractory (r/r) B-cell malignancies. CD19-41BBz chimeric antigen receptor T cell (CAR T) therapy was approved for r/r B-cell ALL in children in 2017 (tisagenlecleucel; Kymriah) based

on extremely high response rates and durable remissions in approximately 50% of children with heavily r/r ALL. Relapse following CAR19 therapy in B-ALL can be broadly categorized as CD19⁺ or CD19⁻, and the relative proportion has varied widely⁸. Efforts to decrease CD19⁻ relapse have focused on either targeting alternative antigens or on developing multi-antigen targeting. At Stanford, we have focused on combinatorial targeting using a CD19/22-BBz-CAR. In two Phase I trials conducted under the same IND, we have enrolled over 45 adults with either B-ALL or lymphoma, and over 10 pediatric patients using the autologous CD19/22.BB.z-CAR following standard cyclophosphamide/fludarabine lymphodepletion. The recommended Phase II dose (RP2D) for CD19/22-BBz-CAR in adult B-ALL was defined as 3×10^6 CAR T cells/kg⁹. This experience has provided significant data demonstrating the safety of this therapy and the feasibility of delivering this product to patients with r/r B cell malignancies. In our initial trials of autologous CD19/22.BB.z-CAR, CAR T products meeting predefined release criteria were successfully manufactured in 100% of patients. The CD19/22-BBz-CAR was very well tolerated with only one patient experiencing greater than Grade 2 CRS (Grade 4 CRS) and 3 patients experiencing Grade 3 or 4 neurotoxicity; all fully resolved with standard treatment. CRS occurred with median onset one day following infusion (range 0-13) and lasted a median of 4 days (range 1-12). The CD19/22-BBz-CAR induced responses in 100% of adults with r/r B-ALL, with 82% experiencing a CR (all MRD negative by flow cytometry). CAR T cell expansion and persistence kinetics were typical of BBz-CARs. As has been demonstrated across a series of CAR T cell trials in adult B-ALL¹⁰, relapses were common and durable remission often required consolidative allo-HCT.

1.2.4 Data on CARs administered following allo-HCT

1.2.4.1 Preclinical Data

In murine models, investigators assessed the potential of donor-derived 19-28z CAR T cells to prevent relapse following allo-HCT. They hypothesized that the adoptive transfer of donor-derived 19-28z CAR T cells after an allograft can enhance GVT activity with minimal GVHD activity in allo-HCT recipients. For these studies, they generated retroviral vectors encoding mouse CD19-specific CARs: CD19-m28z, which signals through CD28 and CD3 ζ endodomain, and a control vector, CD19 delta, a tail-less CAR lacking the CD3 ζ signaling domain. To assess the anti-tumor capacity of 19-28z CAR T cells in vivo, they transferred the transduced B6 (C57BL/6J) donor T cells into CD19 positive lymphoma bearing BALB/c recipients of a B6 allograft. Recipients of CD19 delta CAR T cells succumbed to GVHD, whereas recipients of 19-28z CAR T cells had significantly less GVHD and cleared their lymphoma, resulting in significantly improved tumor-free survival. Hence, these preclinical models demonstrated that the administration of allogeneic CD19-specific CAR T cells mediate anti-tumor activity and do not induce an increase in GVHD¹¹.

1.2.4.2 Clinical Data

Donor CAR T cells have also been administered to patients following allo-HCT, primarily for the indication of disease relapse. Kochenderfer et. al. reported outcomes in patients who relapsed despite allo-HCT and standard donor lymphocyte infusion. In this study, 10 patients were treated with a single infusion of donor-derived 19-28z CAR T cells and 3 of these patients had regressions of their malignancies. None of the 10 patients developed GVHD¹².

Cruz et. al. utilized a unique strategy to administer donor-derived 19-28z CAR T cells after allo-HCT. They engineered viral-specific donor-derived T cells to express a CD19-specific CAR. Eight

patients were treated without infusion-related toxicity and none of the patients developed GVHD. Two of the 6 patients with relapsed disease had an objective response, and the 2 patients who received cells while in remission remained in remission. No lymphodepletion was given prior to the infusions and some patients received the cells long after allo-HCT (latest, 13 years)¹³.

Finally, a review of the literature assessed patients treated with donor CD19-targeted CAR T cells in the setting of relapse following allo-HCT. Data from this analysis found that of the 132 patients assessed, four percent (n=5) developed acute GVHD and three percent (n=4) developed chronic GVHD¹⁴.

1.2.5 Background on rationale to administer Orca-T followed by donor 19-22-BBz CAR T cells for patient with ALL

Retrospective analysis of adults with B-cell ALL treated at our center found that 102 patients received their first allo-HCT with myeloablative conditioning from 2014 to 2021. For the entire cohort, outcomes at 4 years found an event-free survival of 54.9% (95% confidence interval (CI), 44.5-67.6), GVHD- and relapse-free survival of 22.3% (95% CI, 14.5-34.3), and overall survival of 66.2% (95% CI, 56-78.3).

1.2.6 Importance of the Clinical Trial

These data suggest that novel approaches in combination with myeloablative conditioning followed by allo-HCT may be needed to improve patient outcomes. Given the expertise of our center with T_{reg}, HSPC, and T_{con} (Orca-T) and CD19/CD22-CAR T cells, we propose that patients receive a myeloablative conditioning regimen and Orca-T followed by the addition of donor CD19/CD22-CAR T cells as a novel approach to improve outcomes in patients with high-risk B-cell ALL.

1.3 Potential Risks and Benefits

1.3.1 Safety Considerations for CD19/CD22 CAR Therapy

Please see Sections 1.3.5 and 1.3.6 for specific information on CAR-mediated toxicities.

1.3.2 Risk of chemotherapy:

Toxicities resulting from fludarabine and cyclophosphamide in the doses proposed in the current study are well known and are what have been used in the prior anti-CD19 CAR, anti-CD22 CAR, and anti-CD19/CD22 CAR protocols. Such a preparative regimen is designed to decrease the number of endogenous T cells, including T regulatory cells that may otherwise suppress anti-CAR T cell cytotoxicity, and to induce increased availability of homeostatic cytokines thereby allowing for better engraftment of the transferred CAR T cells. The dose limiting toxicity for both fludarabine and cyclophosphamide is myelosuppression. However, myelosuppressive effects are expected to be transient using the doses proposed. Other toxicities including fever, nausea, vomiting, stomatitis, diarrhea, anorexia, edema, skin rashes, myalgias, headache, agitation, and fatigue should be easily managed with appropriate supportive care. Sterile hemorrhagic cystitis occurs in about 20% of subjects who receive cyclophosphamide. Hemorrhagic cystitis is unlikely given the relatively low dose of cyclophosphamide administered in this trial as well as the continuous intravenous infusion of normal saline that will be used prophylactically as a uroprotective agent. Tumor lysis syndrome (TLS) following fludarabine and cyclophosphamide administration can

occur in subjects with advanced bulky disease. To prevent this, subjects who are at high risk of TLS, at the investigator's discretion, will be prescribed allopurinol and appropriately hydrated with close monitoring, by uric acid testing, for the development of TLS. Finally, opportunistic infections (e.g., protozoan, viral, fungal, and bacterial) have been observed post-fludarabine and cyclophosphamide, especially in heavily pre-treated individuals. Subjects will receive appropriate antimicrobial prophylaxis (e.g., Bactrim for PCP and acyclovir for HSV and VZV prophylaxis) during treatment and for a minimum of 6 months following treatment.

1.3.3 Risk of Autoimmunity:

Autoimmune toxicity is a risk of adoptive cell therapy trials for cancer and can occur if the transferred populations recognize the target antigen on normal tissues. Thus far, on-target, off-tumor toxicity of both CD19-CAR and CD22-CAR T cells has been restricted to B cell aplasia, which can be managed with immunoglobulin replacement therapy. Given that the CD19/CD22-CAR T cells tested in this study incorporate scFvs that have already been tested in clinical trials, it is unlikely that unexpected autoimmune toxicity will occur, but subjects will be monitored closely for the occurrence of unexpected toxicity. However, if autoimmunity is observed, then appropriate supportive care and modification to the clinical trial will be instituted.

1.3.4 Risks of Gene Therapy:

Risks of gene therapy include insertional mutagenesis or emergence of replication-competent lentivirus (RCL). While insertional mutagenesis is theoretically possible using retroviral vectors, this has only been observed in the setting of infants treated for X-SCID using retroviral vector-mediated gene transfer into CD34+ bone marrow stem cells. In the case of retroviral or lentiviral vector-mediated gene transfer into mature T-cells, there has been no evidence of long-term toxicities associated with these procedures. Even though clinical data currently available suggest that the introduction of lentiviral vectors transduced into mature T-cells is a safe procedure, continued follow-up of all gene therapy subjects will be performed as required. The proposed protocol follows all current FDA guidelines regarding testing and follow up of subjects receiving gene transduced cells. Similarly, the viral vectors used have been engineered to minimize the risk of emergence of replication competent lentivirus, but subjects will be monitored according to Recombinant DNA Advisory Committee guidelines for several years following receipt of this therapy. In the case of the development of a second malignancy, all efforts will be made to determine whether replication competent lentivirus has emerged.

1.3.5 Risk of Cytokine Release Syndrome:

Cytokine release syndrome occurs in the setting of a variety of immunotherapies including following antibody infusions and following adoptive cell therapy. The clinical picture, now described by many groups, closely resembles infection/sepsis, with fever being the hallmark sign. The pathophysiology is related to excess cytokine levels, released either directly from the adoptively transferred cells or indirectly induced by the immune activation. Severe cytokine release syndrome can be associated with signs and symptoms of hemophagocytic lymphohistiocytosis and macrophage activation syndrome (HLH-MAS).

Grading and management of CRS for subjects in the dose escalation cohort will follow the guidelines set forth by ASTCT Consensus Criteria¹⁵ (see **Section 12.3, Appendix C**), which includes diligent supportive care and search for infection, with immunosuppression using

anti-IL6R monoclonal antibodies and/or corticosteroids reserved for more severe cases. Using such a risk adapted system for management of CRS has diminished morbidity and mortality associated with this syndrome.

Forty-nine experts from experts in immune effector cell therapies, including CAR T therapy met at a meeting supported by the American Society for Blood and Marrow Transplantation (ASBMT) and reached consensus on the grading definitions for immune effector cell-associated CRS¹⁵ contained in **Section 12.3, Appendix C**.

1.3.6 Risk of Immune Effector Cell-Associated Neurotoxicity Syndrome (ICANS)

Neurotoxicity is also observed in a significant minority (20-30% in most series) of subjects¹⁵⁻¹⁷ with toxicity ranging from mild to severe. Symptoms tend to be more diverse than observed with CRS¹⁵. The pathobiology of CD19-CAR associated neurotoxicity is not fully understood, but the clinical syndrome is associated with increased expansion of CAR T cells and identification of CAR T cells in the cerebrospinal fluid. Neurotoxicity may be seen more frequently in subjects with CNS leukemia¹⁶. However, subjects without documented CNS leukemia or lymphoma can also develop symptoms of neurotoxicity that range from mild to severe. Hence, no direct association between CNS disease burden and neurotoxicity severity has been observed in the context of CD19-CAR therapies for leukemia¹⁶. The syndrome typically manifests clinically as tremors, dysgraphia, impaired attention, confusion, aphasia and/or dysmetria, and occasionally seizures. Expressive aphasia appears to be a characteristic feature. Radiographic changes are variable and the syndrome typically resolves in 1-2 weeks and appears fully reversible in the vast majority of patients. The prevailing hypothesis regarding the pathophysiology of this syndrome is that it reflects non-specific neurotoxic effects of cytokines and/or activated T cells, rather than a direct on-target effect of CAR T cells. Indeed, the dose limiting toxicity of IL-6 when administered in a Phase 1 trial was neurotoxicity and transient aphasia was observed¹⁸. Lethal neurotoxicity has occurred in <1% of patients treated with CD19-CAR T cells and is associated with cerebral edema and herniation in patients with severe cytokine release syndrome.

In summary, the ASTCT consensus group convened in 2018 defined ICANS as “a disorder characterized by a pathologic process involving the central nervous system following any immune therapy that results in the activation or engagement of endogenous or infused T cell and/or other immune effector cells. Symptoms or signs can be progressive and may include aphasia, altered level of consciousness, impairment of cognitive skills, motor weakness, seizure and cerebral edema”¹⁵.

While immunosuppression appears to effectively reverse cytokine release syndrome, treatment with anti-IL6R for neurotoxicity does not appear to be effective and may even lead to transient worsening due to elevations in circulating IL-6 induced by the mAb. Corticosteroids may have some beneficial effects, but controlled trials have not been undertaken. Therefore, the true effectiveness of corticosteroids for management of severe neurotoxicity remains unclear.

Furthermore, we postulate that the risk of fatal or very severe neurotoxicity may be reduced with a 4-1BB based on the experience thus far that suggests that 4-1BB costimulatory domain based CARs do not typically undergo as rapid expansion as those using a CD28 costimulatory domain. The CAR utilized in this study will incorporate a 4-1BB costimulatory domain.

Subjects will be monitored carefully for neurotoxicity throughout this study. Additionally, we will also have prospective neurologic evaluations performed on this protocol to provide additional input and insight into the treatment and management of the subjects who do develop

neurotoxicity. All subjects will be treated prophylactically with levetiracetam (Keppra), unless contraindicated, and subjects with significant neurotoxicity will be monitored closely and treated aggressively as outlined in section **Section 12.3, Appendix C**.

1.3.7 Potential Benefits

The study investigators will closely monitor the patients throughout the study, and aspects of the medical care that are related to research will be covered by the study. Patients may have the chance to help others get better treatment in the future based upon the knowledge that is gained from the clinical study. Patients will be treated with cell therapy, donor CD19/CD22-CAR T cells, that are not routinely given following the Orca-T infusion.

2 STUDY DESIGN, OBJECTIVES, AND OUTCOMES

2.1 Study Design

2.1.1 Overall Design

The intervention model is a **Phase 1 open-label, dose-escalation, single group, non-randomized, non-controlled single center** trial.

2.1.2 Primary Purpose of the Study

Purpose: To assess the safety of administering allogenic, donor-derived CD19/CD22-CAR T cells that meet established release specifications in adults with B-cell ALL following a myeloablative conditioning regimen and Orca-T to determine if this will augment graft versus leukemia without increasing acute GVHD or graft failure.

Treatment: In this study patients will receive a myeloablative preparative regimen consisting of chemotherapy +/- total body irradiation. On Day 0, patients will receive a T_{reg} and HSPC infusion. On Day +2, patients will receive an infusion of conventional T cells (T_{con}) followed by infusion of donor CD19/CD22-CAR T cells. We will investigate the addition of donor CD19/CD22-CAR T cells as a strategy to decrease relapse in patients with B-cell ALL.

2.1.3 Study Cohorts / Arms / Groups

The study will consist of three cohorts consisting of 3 to 9 patients per cohort. We will employ a Bayesian dose escalation design for the dosing of the donor CD19/CD22-CAR T cells. There are three planned dose levels: 1×10^6 , 2×10^6 and 3×10^6 donor CD19/CD22 CAR positive T cells per kg ($\pm 20\%$). The starting point is Dose Level 1. If toxicity is noted at Dose Level 1, then the Dose Level -1 is as follows: 0.5×10^6 CAR+ cells/kg. The Bayesian dose escalation design is generated by allowing for the three planned dose levels (1, 2, 3) and the step-down level (-1).

2.1.4 Scientific Justification for Study Design

The scientific justification for this novel study design is noted below:

- Preclinical¹¹ and clinical¹²⁻¹⁴ findings demonstrate that donor CAR T cells can be administered following allo-HCT without inducing increased GVHD.

- Retrospective analysis of patients with B-cell ALL treated at our center found that 102 patients received their first allo-HCT with myeloablative conditioning from 2014 to 2021. For the entire cohort, outcomes at 4 years found an event-free survival of 54.9% (95% confidence interval (CI), 44.5-67.6), GVHD- and relapse-free survival of 22.3% (95% CI, 14.5-34.3), and overall survival of 66.2% (95% CI, 56-78.3).
- These data suggest that novel approaches in combination with myeloablative conditioning followed by allo-HCT may be needed to improve patient outcomes.

2.1.5 Treatment Assignment

This is an **open-label** Phase I dose escalation study to assess the safety donor CD19/CD22CAR T cells plus Orca-T infusion. Patients with B-cell ALL at high risk for relapse or progression who have a matched-related or unrelated donor will be candidates for enrollment on the study.

2.1.6 Justification for Dose(s)

Targeted therapy with tumor specific T cells has been widely used in cancer therapy. The ability of tumor-specific T cells to eradicate tumor is best illustrated in patients with relapsed chronic myelogenous leukemia (CML) after allo-HCT^{19,20}. In this setting, the adoptive transfer of donor T cells alone is often sufficient to induce a second remission through a graft versus leukemia (GVL) mechanism^{19,20}. Investigators have administered various dose levels of donor leukocyte infusions (DLI), and the DLI has been infused at varying time points post-allo-HCT. In one notable study, investigators treated 38 patients with various hematologic malignancies with a T-cell depleted transplant. Of the 38 patients in the study, 26 patients received DLI of 2×10^6 cells/kg on Day 30 and 5×10^7 cells/kg on Day 45 (schedule 1). The overall probability of grade $\geq$ II aGVHD developing was 31.5%, with a 15.5% probability of aGVHD occurring after DLI. The remaining 12 patients received DLI at 10^7 T cells/kg on Day 30 (schedule 2), and the probability of grade $\geq$ II aGVHD was 100%²¹. This data highlights that the dose and timing of the administration of DLI are both important factors to consider in regard to their GVHD potential. To minimize the potential for GVHD, we propose to administer a single dose of donor CD19/CD22-CAR T cells at 10^6 cell/kg in the setting of T_{regs} and tacrolimus for GVHD prophylaxis.

Based on data from the autologous CD19/CD22 study, the recommended Phase II dose (RP2D) for CD19/22-BBz-CAR T cells in adult B-ALL was defined as 3×10^6 CAR T cells/kg⁹. Hence, we selected this dose as our target for the Dose 3 Cohort. Dose 1 and Dose 2 Cohorts will receive lower doses of donor CD19/CD22-CAR T cells so that we can monitor for the risk GVHD and graft failure.

2.1.7 End of Study Definition

A clinical trial is considered completed when participants are no longer being examined or the last participant's last study visit has occurred.

A participant is considered to have completed the study if he or she has completed all phases of the study including the last visit or the last scheduled procedure shown in the Schedule of Events at Section 6.3. The end of the study is defined as completion of the last visit or procedure shown in the Schedule of Events in the trial globally.

2.2 Objectives

Endpoints are discussed in **Section 10 Statistical Considerations**.

2.2.1 Primary Objective

- Assess the feasibility of manufacturing and the safety of administering allogeneic, donor CD19/CD22-CAR T cells that meet established release specifications and to determine the MTD in adults with B-cell ALL following a myeloablative conditioning regimen and purified regulatory T-cell (T_{reg}) plus CD34+ hematopoietic progenitor cells ("Orca-T").

2.2.2 Secondary Objectives

- Determine the cumulative incidence of relapse or progression of disease as well as non-relapse mortality (NRM) following donor CD19/CD22-CAR T cells plus Orca-T infusion.
- Assess the rate of secondary graft failure following donor CD19/CD22-CAR T cells plus Orca-T infusion.
- Analyze progression-free (PFS) and overall (OS) survival following a donor CD19/CD22-CAR T cell infusion plus Orca-T infusion.
- Evaluate the incidence of greater than Grade 3 infectious disease complications

2.3 ClinicalTrials.gov Registration, Outcomes, and Results

2.3.1 Data Sharing Statement

Pursuant to the International Committee of Medical Journal Editors (ICMJE), the following statement, regarding sharing of individual patient data (i.e., participant-level data) generated by interventional clinical trials, is provided.

It is not planned that individual participant data, including data dictionaries, will be made publicly available. Individual participant study data judged by the investigator to be pertinent to a participant's understanding of their medical condition and treatment options will be shared with that participant. Otherwise, and as such, details of what, or when, or by what mechanism, data and/or documents will be shared, who would share such data, or for what purposes or analyses, are not available or not applicable.

Although not individual participant data, pursuant to the requirements of [42CFR§11.48\(a\)\(5\)](#) (if applicable), the final IRB-approved protocol document with statistical analysis will be made available in the ClinicalTrials.gov results record.

2.3.2 Outcome Measures for ClinicalTrials.gov Results Reporting

Per CFR Part 11, this study is not an applicable drug clinical trial for results reporting due to being a phase 1 clinical investigation.

3 SUBJECT SELECTION

3.1 Eligibility Criteria and Participant Eligibility Checklist

Inclusion and Exclusion Criteria are provided on the Eligibility Checklist which may be extracted from this document for use in screening potential subjects.

The Participant Eligibility Checklist must be completed in its entirety for each subject prior to registration (see Section 3.4 Registration / Enrollment). The completed, signed, and dated checklist is retained in the subject's record. Screening results will be collectively documented on the Study Participant Log.

Participant Eligibility Checklist

I. Protocol Information

Protocol Title:	Phase 1 Trial Evaluating the Safety of Myeloablative Conditioning, Orca-T, and Allogeneic, Donor-Derived CD19/CD22-CAR (Chimeric Antigen Receptor) T cells in Adults with B-cell Acute Lymphoblastic Leukemia (ALL)
OnCore number:	BMT378
Principal Investigator:	Lori Muffly, MD, MS

II. Subject Information

Subject name / Unique ID:		/
Gender	☐ Male	☐ Female

III. Eligibility Criteria

3.1.1 Recipient Inclusion Criteria

Prospective Participant Must Meet ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
1. Disease Status of B-cell ALL (one of the following criteria must apply) a. Subjects in CR must have a history of chemotherapy refractory disease defined as progression OR stable disease after one line of chemotherapy, OR relapsed disease after achieving prior CR, OR a history of residual disease in the peripheral blood or bone marrow (by flow cytometry, PCR, FISH, or next generation sequencing) following induction or consolidation therapy OR must have other high risk ALL features per the NCCN Guidelines (Version 1.2022), Acute Lymphoblastic Leukemia (Appendix I) b. Subjects with active ALL (defined as $\geq 5\%$ bone marrow blasts, circulating blasts, or extramedullary disease) are eligible.	☐	☐	
2. Age ≥ 18 and ≤ 65 years (i.e., from age 18 to < 66 years old) at the time of enrollment	☐	☐	
3. Eastern cooperative oncology group (ECOG) performance status of 0, 1, or 2; or Karnofsky $\geq 60\%$	☐	☐	

Prospective Participant Must Meet ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
4. CD19 expression is required any time since diagnosis. CD19 expression may be detected by immunohistochemistry or by flow cytometry. The choice of whether to use flow cytometry or immunohistochemistry will be determined by what is the most easily available tissue sample in each subject. In general, immunohistochemistry will be used for lymph node biopsies, flow cytometry will be used for peripheral blood and bone marrow samples. Patients receiving prior CD19 CAR T cell or blinatumomab are eligible if there is no documented history of CD19 negativity on the malignant cells.	☐	☐	
5. Subjects must have an HLA matched related donor or unrelated willing to undergo unstimulated apheresis for T cell collection for CAR T cell generation followed by GCSF mobilized apheresis Orca-T generation.	☐	☐	
6. Matched related or unrelated donor who is an 8/8 match for HLA-A, -B, -C, and -DRB1, all typed using DNA-based high-resolution methods	☐	☐	

Prospective Participant Must Meet ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
7. Subjects must have adequate organ function measured by: ○ Cardiac: Cardiac ejection fraction at rest $\geq$ 45% ○ Hepatic: ○ Total bilirubin $<$ 2 times upper limit of normal (ULN) (patients with Gilbert's syndrome may be included where hemolysis has been excluded and with approval of the medical monitor) ○ ALT/AST $\leq$ 3 times ULN ○ Renal: Calculated creatinine clearance $\geq$ 50 mL/min or creatinine $<$ 2.0 mg/dL ○ Pulmonary: Diffusing capacity of the lung for carbon monoxide (DLCO) (adjusted for hemoglobin) $\geq$ 50% ○ CNS: Subjects with CNS involvement are eligible as long as there are no overt signs or symptoms that in the evaluation of the investigator would mask or interfere with the neurological assessment of toxicity.	☐	☐	
8. Negative serum or urine beta-HCG test in females of childbearing potential within 3 weeks of registration.	☐	☐	
9. Subjects of childbearing or child fathering potential must be willing to practice birth control from the time of enrollment on this study and for four (4) months after receiving the preparative conditioning regimen.	☐	☐	

Prospective Participant Must Meet ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
10. Must be able to give informed consent. Legal authorized representative (LAR) is permitted if subject is cognitively able to provide verbal assent.	☐	☐	

3.1.2 Recipient Exclusion Criteria

Prospective Participants Must <u>NOT</u> Meet <u>ANY</u> of These Exclusion Criteria	Yes	No	Supporting Documentation *
1. History of other malignancy, unless disease free for at least 3 years. At the discretion of the Principal Investigator, subjects in remission for 1-2 years prior to enrollment may be deemed eligible after considering the nature of other malignancy, likelihood of recurrence for one year following therapy, and impact of prior treatment on risk of CD19/CD22-CAR T cells and Orca-T infusion. Subjects in remission <1 year are not eligible. The following exceptions apply: a. Nonmelanoma skin cancer or carcinoma in situ (e.g., cervix, bladder, breast) is eligible. b. Hormonal therapy in subjects in remission >1 year will be allowed.	☐	☐	
2. Patients who have undergone a prior allogeneic or autologous stem cell transplant.	☐	☐	

Prospective Participants Must <u>NOT</u> Meet <u>ANY</u> of These Exclusion Criteria	Yes	No	Supporting Documentation *
3. Recipient positive anti-donor HLA antibodies against a mismatched allele in the selected donor determined by either: ○ a positive crossmatch test of any titer (by complement-dependent cytotoxicity or flow cytometric testing), or ○ the presence of anti-donor HLA antibody to any of the following HLA loci: HLA-A, -B, -C, -DRB1, -DQB1, -DQA1, -DPB1, or -DPA1, with mean fluorescence intensity (MFI) >1000 by solid phase immunoassay	☐	☐	
4. Presence of fungal, bacterial, viral, or other infection that is uncontrolled. Simple UTI and uncomplicated bacterial pharyngitis are permitted if responding to active treatment.	☐	☐	
5. Known history of infection with any of the following: a. HIV b. Hepatitis B (HBsAg positive) ** c. Hepatitis C virus (anti HCV positive) ** ** A history of hepatitis B or hepatitis C is permitted if the viral load is undetectable per quantitative PCR and/or nucleic acid testing.	☐	☐	
6. Currently receiving corticosteroids or other immunosuppressive therapy. Topical corticosteroids or oral systemic corticosteroid doses less than or equal to 10 mg/day are allowed.	☐	☐	

Prospective Participants Must <u>NOT</u> Meet <u>ANY</u> of These Exclusion Criteria	Yes	No	Supporting Documentation *
7. Planned pharmaceutical in vivo or ex vivo T cell depletion, e.g., post-transplant cyclophosphamide (Cy), peri-transplant anti-thymocyte globulin (ATG), or alemtuzumab. For patients that have previously been exposed to a T cell-depleting agent, a 5-half-life washout of the agent must occur prior to planned Transplant Day 0	☐	☐	
8. Hyperleukocytosis ($\geq 50,000$ blasts/ μL) or rapidly progressive disease that in the estimation of the investigator and sponsor would compromise ability to complete study therapy.			
9. History of myocardial infarction, cardiac angioplasty or stenting, unstable angina, or other clinically significant cardiac disease within 12 months of enrollment.	☐	☐	
10. Pregnant or breast feeding	☐	☐	
11. Patients with known autoimmune disease requiring the use of systemic immunosuppressive therapy within the last year	☐	☐	
12. Presence of a seizure disorder, cerebrovascular ischemia/hemorrhage, dementia, cerebellar disease, or any autoimmune disease with CNS involvement that in the judgment of the investigator may impair the ability to evaluate neurotoxicity.	☐	☐	

Prospective Participants Must <u>NOT</u> Meet ANY of These Exclusion Criteria	Yes	No	Supporting Documentation *
13. Any medical condition that in the judgement of the investigator is likely to interfere with assessment of safety or efficacy of study treatment	☐	☐	

3.1.3 Donor Inclusion Criteria

Prospective Participant Must Meet ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
1. Age ≥ 16 and ≤ 75 years at time of enrollment	☐	☐	
2. Karnofsky performance status of $\geq 70\%$ defined by institutional standards	☐	☐	
3. Willing to donate for two separate apheresis collections (T cells and PBSC)	☐	☐	
4. HLA match to the patient in either one of the following classifications: a. Matched related or unrelated donor who is an 8/8 match for HLA-A, -B, -C, and -DRB1, all typed using DNA-based high-resolution methods	☐	☐	
5. Seronegative for HIV-1 RNA PCR; HIV 1 and HIV 2 Ab (antibody); HTLV-1 and HTLV-2 Ab; PCR+ or sAg negative (surface antigen for hepatitis B); negative for the Treponema pallidum antibody Syphilis screen; and negative for HIV-1 and hepatitis C by nucleic acid testing (NAT) within 40 days of donor apheresis procedures.	☐	☐	

Prospective Participant Must Meet ALL these Inclusion Criteria to be Eligible	Yes	No	Supporting Documentation *
6. In the case that T pallidum antibody tests are positive, donors must: • Be evaluated and show no evidence of syphilis infection of any stage by physical exam and history • Have completed effective antibiotic therapy to treat syphilis • Have a documented negative non-treponemal test (such as RPR) or in the case of a positive non-treponemal test must be evaluated by an infectious disease expert to evaluate for alternative causes of test positivity and confirm no evidence of active syphilitic disease	☐	☐	
7. Ability to understand and the willingness to personally sign the written IRB-approved informed consent document.	☐	☐	

3.1.4 Donor Exclusion Criteria

Prospective Participants Must <u>NOT</u> Meet ANY of These Exclusion Criteria	Yes	No	Supporting Documentation *
1. Evidence of active infection	☐	☐	
2. Seropositive for HIV-1 or -2, HTLV-1 or -2	☐	☐	
3. Positive serologic or PCR test results indicating acute or chronic HBV infection: Donors whose HBV infection status cannot be determined conclusively by serologic test results (www.cdc.gov/hepatitis/hbv/pdfs/serologicchartv8.pdf) must be negative for HBV by PCR to be eligible for study participation	☐	☐	

Prospective Participants Must <u>NOT</u> Meet <u>ANY</u> of These Exclusion Criteria	Yes	No	Supporting Documentation *
4. Potential for Zika virus infection as defined as any of the following: a. Medical diagnosis of Zika virus infection in the past 6 months b. Residence in, or travel to, an area with active Zika virus transmission within the past 6 months. c. Unprotected sex within the past 6 months with a person who is known to have either of the risk factors listed above Donors determined to be ineligible based on the results of Zika virus screening may be determined to be eligible if: d. The donor has no signs or symptoms consistent with active Zika virus infection and e. Either of the following is true: ○ The donor is a first-degree or second-degree blood relative of the recipient ○ Urgent medical need, meaning no comparable human cell product is available and the recipient is likely to suffer death or serious morbidity without the human cell product, as attested by the Investigator.	☐	☐	
5. Medical, physical, or psychological reason that would place the donor at increased risk for complications from growth factor or leukapheresis.	☐	☐	

* All subject files must include supporting documentation to confirm subject eligibility.

V. Statement of Eligibility

By signing this form of this trial I verify that this recipient and donor are:

☐ eligible for participation in the study ☐ ineligible for participation in the study

Study Coordinator printed name:	Date:
Signature:	
Investigator printed name:	Date:
Signature:	
Triple-check reviewer printed name:	Date:
Signature:	

3.1.5 Lifestyle Considerations

During this study, participants are asked to:

- Refrain from driving for 8 weeks post-CAR T infusion given the potential for ICANS
- Practice birth control for 12 months
- Stay local to treating center
- Refrain from operating heavy duty equipment guns etc.
- Refrain from gardening due to risk of infection

3.1.6 Screen Failures

Participants who consent to participate in the clinical trial, but who do not meet 1 or more Eligibility Criteria during the screening procedures are considered Screen Failures. Screen Failures will not be considered enrolled subjects, although they **WILL** be tracked on the Screening Log (with reason for ineligibility) and in the OnCore study management system.

3.2 Recruitment Procedures

3.2.1 Recruitment

Potential participants may be identified by the following methods:

- Review of the medical records of new or existing oncology patients for potential subjects who meet eligibility criteria.
- Stanford physicians may refer patients to the study for screening. A study investigator and/or study coordinator will discuss the study with the participant during a patient visit or with a visit scheduled for the purpose of recruitment to the study. This study will be listed on the Stanford Clinical Trials website and ClinicalTrials.gov. Patients of a particular clinic, physician, or other caregiver who is NOT a study investigator will be contacted or approached only after their physician has previously notified the participant about the study and obtained approval for contact by the study team.
- Self-referral by subject after viewing study listing on ClinicalTrials.gov or the Stanford Clinical Trials website.
- External physicians may refer patients to the study for screening.
- ALL is over-represented in males. So, we will aim for our accruals to include at least 35% women. ALL is also at higher incidence in Hispanic patients. So, we will also aim for at least 35% of our accruals to be in Hispanic patients. Additionally, we will aim to accrue at least one African-American patient, given that this disease is relatively rare in African-American populations.
- Participants will not be compensated or provided any incentives (e.g., vouchers, gift cards) for study participation.

3.3 Informed Consent Process

The BMT attending physician discusses the rationale, logistics, risks, and benefits of the study with the subject. Alternative therapies are discussed. The subject is given a copy of the consent form to take home and read.

Then, a meeting is scheduled with a specialized trained physician, or registered nurse or research study staff to review the rationale, logistics, treatment plan, risks, benefits, and consent form. The subject's bill of rights is reviewed with an emphasis placed on voluntary participation. Subjects are given time to ask questions and do not need to sign the consent form until they are ready to proceed.

Sufficient time is scheduled and devoted to the consent discussion with a clinical nurse specialist or research nurse educator. The BMT attending physician is available to discuss the study with the subject at any time.

During the consent teaching, the physician, nurse, or research staff member frequently asks questions of the subject to assess their understanding and a consent note is written documenting the subject's understanding.

For subjects that are not fluent in English, the consent procedure is performed with an interpreter present (or via telephone/video), or a signer for those that are hearing impaired. Consents are translated into the appropriate language as needed or a short form process is used.

Study participants will provide written informed consent prior to the conduct of any study-specific procedures in accordance with institutional policies.

Subjects are evaluated by a physician, nurse, and social worker. Any member of the team can request a psychiatric evaluation if a concern of competency to provide consent is raised.

3.4 Registration and Enrollment

3.4.1 Subject Registration Procedures

All subjects who provide informed consent for this study will be registered in the Stanford OnCore Enterprise Research System ("OnCore") database within 5 days of the date of consent, regardless of the outcome of any eligibility screening. After the subject is determined to be completely eligible to participate in the study, the OnCore sequence number, a unique subject identifier, will be sequentially assigned.

3.4.2 Subject Enrollment Procedures

For this study, a subject is enrolled on the study when registered per **Section 3.4.1**; has met eligibility per the screening procedures; and otherwise, has been accepted into the study.

All eligibility criteria of transplant recipients are to be satisfied within 60 days of initiation of the recipient's conditioning regimen.

All eligibility criteria of transplant donors are to be satisfied within 45 days of the donor leukapheresis procedures.

Initiation of study treatment will be the initiation of the conditioning regimen (infusion recipients) or T cell apheresis (cellular donors).

See Pre-Treatment Criteria in Section 5.

4 STUDY INTERVENTIONS

4.1 Investigational Drug: CD19/CD22-CAR T cells

4.1.1 Name(s)

Allogeneic donor-derived T-cells transduced with bivalent lentiviral vector (CD19/CD22-BBz) chimeric antigen receptor (CAR); following preparative chemotherapy regimen.

4.1.2 Dose and Administration

CD19/CD22CAR T cells will be administered at a dose of CAR+ cells/kg body weight via IV administration as per [Table 5 CD19/CD22-CAR T cell Dose](#) Schema

4.1.3 Regulatory Status of CD19/CD22-CAR T cells

Donor CD19/CD22-CAR T cells are not approved for marketing in the United States for any indication, and all uses are investigational. Regulatory authorization to conduct this study is pursuant to IND 28621 submitted by Melody Smith, MD, MS.

4.1.4 CD19/CD22-CAR T Cells Preparation, Handling, Storage, and Accountability

Donor CD19/CD22CAR T cells will be generated from fresh or cryopreserved PBMCs under GMP conditions in a cell manufacturing facility using Miltenyi CliniMACS Prodigy® system. Apheresis products from participating healthy donors will be sent to the manufacturing facility through Stanford Bone Marrow Transplant Department's Cellular Therapeutics Facility (CTF); final cell products will be received, and the final formulated product will be distributed by the CTF.

Any prepared cells not required for the first infusion or for research or regulatory purposes (including sufficient QC retention vials for testing cryopreserved product stability at time of additional infusions) will be cryopreserved by standard techniques. Additional product in excess of that needed for infusion may be utilized for research.

Fresh or cryopreserved PBMCs (depending on the timing of apheresis relative to cell culture, subject condition, and scheduling availability) will be used for cell manufacturing. Cells will be required to meet the IND release criteria as outlined in **Table 1 CD19/CD22-CAR Product Rapid Release Criteria**, as well as no evidence for replication competent lentivirus. All procedures will take place using good manufacturing process guidelines.

Additional information on the manufacturer facility information, package label, container system, and formulation are provided in the IND Chemistry and Manufacturing Controls (CMC) section.

Table 1 CD19/CD22-CAR Product Rapid Release Criteria

Test	Criteria
Cell viability ¹	≥ 70%
Total Cell Count ¹	within 20% of target dose level
CD3+%	≥ 70%
CAR+ %	≥ 10%
Endotoxin ¹	< 5 EU/kg
Mycoplasma by PCR ¹	Negative
RCL ²	Negative
Sterility testing ³	Negative

¹Performed on sample from final product, results available at the time of infusion.

²Two samples will be sent to Indiana University GTTL: 1) Real-time PCR testing, results available at the time of infusion; 2) culture-based testing; results not available prior to infusion.

³Gram stain is performed on final product prior to infusion and is available at the time of infusion; 14-Day sterility and 42-Day fungal cultures will be sent from the harvest (Day 7 to 12) drug product. Sample results collected from the final product will not be available before cells are infused into the subject. Cell product will be released based on a 3-5 day preliminary sterility and fungal result.

4.1.4.1 Storage and Stability

Clinical doses are frozen in cryobags in a controlled rate freezer. Clinical product is then transported to and stored in temperature monitored liquid nitrogen freezer, monitored by an alarm system. Up to 2 clinical doses will be frozen per patient, based on cell expansion. The product expiration is within 6 hours post-thaw.

4.1.4.2 Preparation

Cryopreserved cells are thawed at bedside by trained CTF staff and administered over 10 to 20 min +/- 5 minutes.

4.1.4.3 Investigational Agent Ordering

If product release criteria are met, the Laboratory for Cell and Gene Medicine (LCGM) will ship the clinical product to the CTF at an agreed upon date and time, using a liquid nitrogen shipper with temperature monitoring capabilities. A chain of custody and product certificate of analysis will accompany the clinical product from the manufacturing facility to the CTF.

The mailing address and local contact phone number for the drug supplier contact facility is:

Name: Kunjan Kamdar and Arlene Ajoste (QA Associate Directors)

Address: 855 S California Ave, Unit C, Palo Alto, CA, 94304

Phone: K.K. - 408-603-5886; A.A. - 415-309-9566

Email: lcgm-qagroup@lists.stanford.edu

4.2 Investigational Agent: T_{reg}, HSPC, and T_{con} (Orca-T)

4.2.1 Name

Purified donor-derived regulatory T cells (T_{reg}), CD34+ hematopoietic stem and progenitor cells (HSPC), and conventional T cells (T_{con}) (the three products make “Orca-T”).

4.2.2 Formulation, Dose, and Administration

Dose consisting of $\geq 2 \times 10^6$ cell/kg HSPC and $\geq 2 \times 10^6$ cells/kg T_{reg} is based on recipient actual body weight rounded to the nearest kilogram (as assessed during screening). Per institutional standard practice, an appropriate long-term central venous access is placed for administration.

4.2.3 Regulatory Status of Orca-T

Orca-T is not approved for marketing in the United States for any indication, and all uses are investigational. Regulatory authorization to conduct this study is pursuant to IND 18873 held by Orca Bio, Inc.

4.2.4 Orca-T Preparation, Handling, Storage, and Accountability

Preparation and storage of the investigational products Orca-T will take place in the facilities described within IND 18873 and released according to compliance with the manufacturing processes and specifications specified within IND 18873.

As necessary, final formulated drug product will be transported from the designated facilities to the BMT-CTF and distributed to all subjects.

4.2.4.1 HSPC Release Criteria

Table 2 Release Criteria for Infusion for HSPC Final Formulated Drug Product

	Attribute	Method	Acceptance Criterion
Purity	Viability	Multiparameter Flow Cytometry	$\geq 70\%$
	% Viable HSPC	Multiparameter Flow Cytometry	$\geq 53\%$
	Residual T-Cell Dose	Calculation: Product of Multiparameter Flow Cytometry and CD45 ⁺ Cell Dose	$\leq 50 \times 10^3$ cells/kg
Potency	CD45 ⁺ Cell Dose	Cell Counting by Coulter Principle Coupled with Fluorescence	$\geq 1.6 \times 10^6$ cells/kg
Safety	Sterility	14-Day Sterility	No growth detected
	Sterility	Gram-Stain	No organisms detected
	Endotoxin	Endosafe-PTS Assay	≤ 0.5 EU/mL

4.2.4.2 T_{reg} Release CriteriaTable 3 Release Criteria for Infusion for T_{reg} Final Formulated Drug Product

	Attribute	Method	Acceptance Criterion
Purity	Viability	Multiparameter Flow Cytometry	≥ 70%
	% Viable T _{reg} Cells	Multiparameter Flow Cytometry	≥ 80%
Potency	CD45 ⁺ Cell Dose	Cell Counting by Coulter Principle coupled with Fluorescence	1.6 – 4.0 x 10 ⁶ cells/kg
Safety	Sterility	14-Day Sterility	No growth detected
	Sterility	Gram-Stain	No organisms detected
	Endotoxin	Endosafe PTS Assay	≤ 0.5 EU/mL
Identity / Potency	FoxP3 ⁺ molecular phenotype	Flow Cytometry (Performed at Stanford CTF only)	≥ 80% (FIO*)

* FIO: For information only, reported at the discretion of study PI

4.2.4.3 T_{con} Release CriteriaTable 4 Release Criteria for Infusion for T_{reg} Final Formulated Drug Product

	Attribute	Method	Acceptance Criterion
Purity	Viability	Multiparameter Flow Cytometry / add SOP#	≥ 70%
Potency	Viable T Cell Dose	Calculation: Product of Multiparameter Flow Cytometry and Cell Counting by Coulter Principle coupled with Fluorescence	1.5 – 5.8 x 10 ⁶ cells/kg
Safety	Sterility	14-Day Sterility	No growth detected
	Sterility	Gram-Stain	No organisms detected
	Endotoxin	Endosafe PTS Assay	≤ 2.0 EU/mL

After processing by Orca Bio, Inc, the cellular components (drug products) will be transferred to BMT-CTF, which is the “cell pharmacy” for the Stanford Health Care. The HSPC and T_{reg} drug products will be securely stored at 2 to 8°C in a continuously monitored environment until released for infusion. The T_{con} drug product will be securely stored at no greater than –125°C in a continuously monitored environment until released for infusion. Product labeling will be compliant with ISBT 128 specifications. The BMT-CTF will dispense the cellular components to the respective subjects.

4.2.4.4 Acquisition and Accountability

Description of the SHC facility: The Stanford BMT-CTF is accredited with the Foundation for the Accreditation of Cellular Therapy (FACT) for cell processing requiring more than minimal manipulation; registered with the FDA; and licensed with the California Department of Health Services for both Biologics Manufacturing and Tissue Banking. The facility is operated as an ISO Class 8 facility with an independent HVAC system providing HEPA-filtered air. Laboratory space is divided into an accessioning/testing area and a cell processing suite containing 4 cell processing bays, a cell sorter bay, and a cryopreservation room. The cell processing suite is equipped with 4 biosafety cabinets and other equipment for standard cell processing procedures. The lab is also equipped with instrumentation for product assessments including a hematology analyzer, controlled rate freezers for cell cryopreservation, and freezers for liquid nitrogen vapor phase storage of frozen cellular products. The facility contains two Miltenyi Biotec CliniMACS Plus Systems for cell enrichment by immunomagnetic cell selection.

4.2.4.5 Formulation, Packaging, and Labeling

Components are provided as single dose transfer bags, with an approximate fill volume of 100 mL each for both the T_{reg} and the HSPC components. The T_{con} component will be provided frozen and stored in the vapor phase of liquid nitrogen, in an approximate volume of 15 mL. Each product consists of selected cells, Multiple Electrolytes Injection Type I (USP), and human serum albumin, at a pH of approximately 7.4. The bags and/or primary bag container will have labels bearing the appropriate label text as required by governing regulatory agencies. Additionally, the T_{con} drug product contains Hetastarch and DMSO.

4.2.4.6 Storage and Stability

HSPC and T_{reg} drug products are maintained at 2-8°C in a continuously monitored environment until released for infusion. T_{con} drug product is maintained at no greater than –125°C in a continuously monitored environment until released for infusion.

4.2.4.7 Preparation

All cryopreserved products for infusion will be thawed either in the cell processing facility or bedside on the day of infusion per CTF SOPs. No washing of the thawed cells is planned.

4.2.4.8 Comparator / Control Agent Ordering

Stanford Blood and Marrow Transplant / Cellular Therapeutics Facility (BMT-CTF);
Room H1353
Stanford University Medical Center / Stanford Health Care
300 Pasteur Dr
Palo Alto, CA 94305

5 TREATMENT PLAN

5.1 Donor Participation Plan

Potential donors will be approached for the study after the decision to proceed with transplantation is made and a suitable HLA-matched related or unrelated donor (8/8) is identified.

Donor study procedures, including informed consent, may be overseen at the investigator's site [e.g., related donors who reside near the investigator's site or unrelated donors overseen by the National Marrow Donor Program (NMDP)].

Donors need to be available and willing to donate for two separate apheresis collections (for manufacturing of CD19/CD22-CAR T cells and for Orca-T). Whenever possible, the donor schedule will occur as described in **Section 5.2**. To accommodate holidays, manufacturing facility availability, and donor preferences, the timing of collection of unmobilized apheresis and manufacture of CD19/CD22-CAR T cells may occur up to 28 days ahead of Day -9. Donor screening procedures should still occur within 45 days of the mobilized collection.

5.1.1 Unrelated Donors

Eligibility Criteria

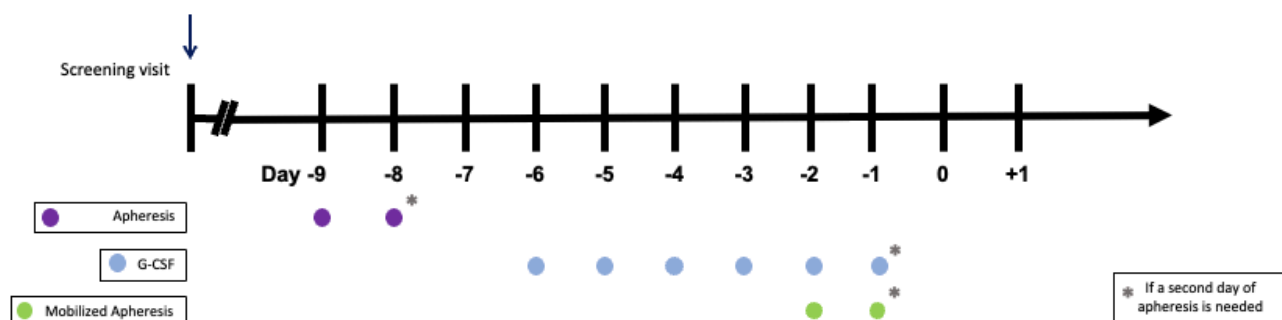
Unrelated donors identified through NMDP will meet eligibility criteria according to NMDP's Standards (NMDP, 2023). Due to NMDP's donor confidentiality and privacy policies, eligibility will be confirmed by the study site based upon NMDP documentation of donor clearance and eligibility that is provided.

Consent Process

Unrelated donors identified through NMDP will be consented by NMDP designee(s). The Stanford BMT team will receive written confirmation of the donor's consent from NMDP. The consent form will be retained by NMDP for confidentiality purposes.

5.2 Donor Schedule

Donor Schematic: CD34+ HSPC Treg graft + donor CD19/CD22-CAR T cell Trial



The asterisks denote the following:

1. For the unmobilized apheresis, if the donor needs a two day collection, the second collection will occur on Day -8.
2. If the donor is scheduled for a mobilized apheresis with two days of collection, then G-CSF will be started in Day -6 and the apheresis will be scheduled on the evening of Day -2 and the morning of Day -1.
3. If the donor is scheduled for a mobilized apheresis with one day of collection, then G-CSF will be started in Day -6 and the apheresis will be scheduled on Day -2.

5.2.1 Donor Premedications

5.2.1.1 Cell Collection for Donor CD19/CD22-CAR T cells

Apheresis procedure, including the machine, collection procedure and supportive care will be in accordance with standardized procedures of Stanford Blood and marrow transplantation (BMT)/Apheresis program.

For the mononuclear cell (MNC) collection, typically process 12-25 L of participant's whole blood. For the hematopoietic progenitor cell assay (HPCA), usually process 3.5-4.5 hours.

5.2.1.2 G-CSF Mobilization

All donors will receive mobilization therapy per institutional guidelines with daily G-CSF. The recommended dose is 10 µg/kg/day SQ (rounded off to the nearest vial size of either 300 or 480 µg). The Mobilization Phase starts on the first day (Day -6 ± 1 day) of administration of G-CSF and continues until Day -2 ± 1 day (or until Day -1 in the event a second day of collection is needed) before the second leukapheresis (Day -2 ± 1 day).

Per institutional standards, plerixafor (e.g., 0.24 mg/kg SC, once) may be administered if recommended by the investigator and/or attending physician to achieve the HSPC dose targets.

While not required per protocol, monitoring donor peripheral blood CD34⁺ cell counts prior to donation is recommended to identify donors that are mobilizing poorly.

5.2.1.3 Cell Collection for Orca-T

All cellular components will be obtained from matched donors mobilized with G-CSF [filgrastim (Neupogen) or equivalent] and collected by apheresis at Stanford Health Care (SHC) as per current institutional allogeneic transplant protocols.

The first leukapheresis will be performed on a continuous flow cell separator according to institutional standards and will start on Day -9 $\pm$ 1 day. The second leukapheresis of the donor will start on Day -2 $\pm$ 1 day with a target of $\geq 3 \times 10^6$ CD34⁺ cells/kg recipient body weight post-selection and a maximum dose of 10×10^6 CD34⁺ cells/kg.

In order to have sufficient cells available for CD34 enrichment and T_{reg} sorting procedures, donors may either undergo large volume apheresis collection on 1 day (Day -2 $\pm$ 1 day) or on 2 consecutive days (Day -2 $\pm$ 1 and Day -1); additional apheresis may be needed to ensure a sufficient T-cell count for above stated reasons; an additional mobilizing agent such as plerixafor (Mozobil) may be used under these circumstances at the discretion of the attending physician.

At the conclusion of each collection at Stanford, cellular products will be picked up from the Apheresis area by the BMT-CTF team and transferred to the cell processing facility. For collection occurring outside of Stanford, products can be transferred on the same day, with the previous days product stored at 4°C or according to institutional practice but not frozen.

The unmobilized collection (Day -9 $\pm$ 1 day) will be kept under refrigeration as a quarantined product in a continuously monitored, secure refrigerator in accordance with apheresis collection storage standards and BMT-CTF SOPs. Prior to being placed in storage, samples for cell count, flow cytometric analysis, and collection sterility testing will be aseptically removed by the cell processing facility staff and submitted to the designated group within the SHC Clinical Laboratory for assessment. After collection, the cellular harvest will be transferred via validated transport methods to the Stanford Laboratory for Cell and Gene Medicine (LCGM) Facility.

The mobilized collection (Day -2 $\pm$ 1 day) will be transferred to the cell processing facility upon completion of apheresis. The product will be sampled in the same manner for assessment. After collection, the cellular harvests will be transferred via validated transport methods to Orca Bio, Inc, facility at 3400 Business Dr (Suite 140) in Sacramento, CA (95820).

5.2.1.3.1 Collection in Case of Graft Failure

There is a risk of graft failure with allogeneic hematopoietic cell transplant procedures. The donors will be notified in the event of graft failure to propose recollection for a second (unmanipulated) transplant procedure.

5.2.1.4 Cell Processing

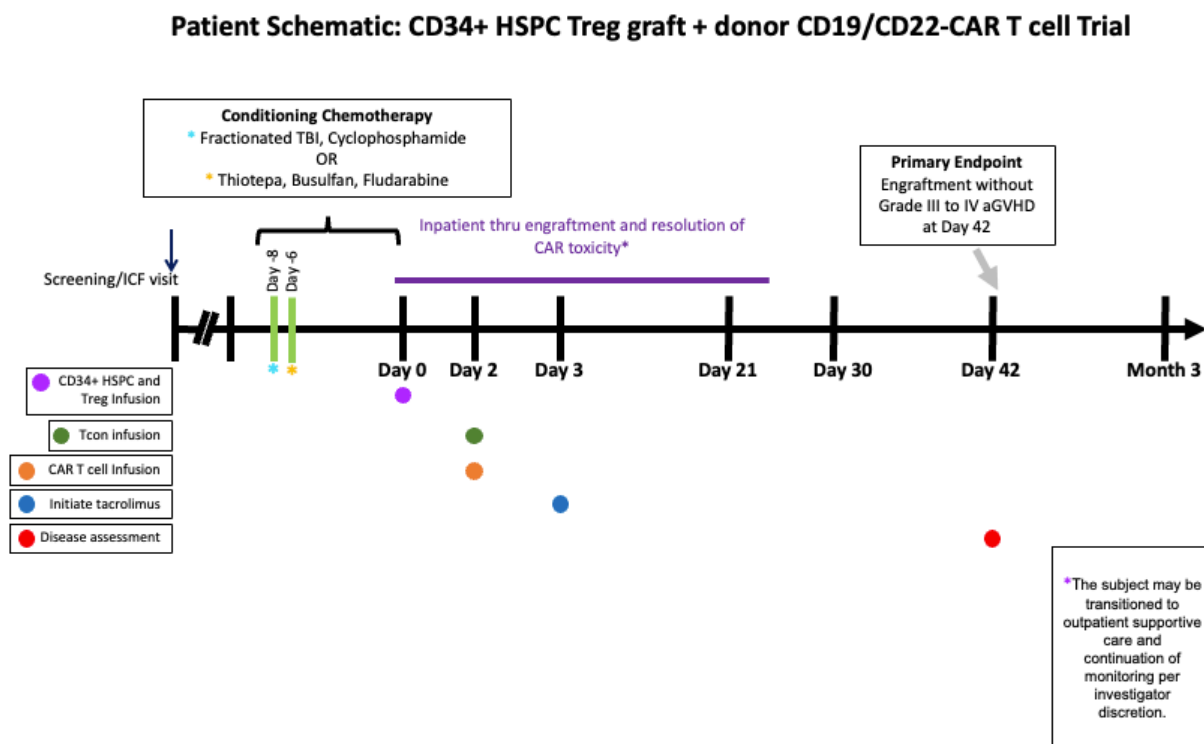
The donor mobilized leukapheresis collections will be the source of the 3 infusions products: conventional T-cells (T_{con}), regulatory T-cells (T_{reg}), and CD34⁺ hematopoietic progenitor/stem cells (HSPC). Upon final formulation, samples from each drug product will be aseptically removed for release testing to assess the product quality.

5.2.1.5 Outcomes Following Processing

After processing by Orca Bio, Inc, the cellular components (drug products), will be transferred to BMT-CTF, which is the “cellular pharmacy” for the Stanford University Medical Center. The BMT-CTF will dispense the cellular components to the respective subjects.

5.3 Recipient Participation Plan

5.3.1 Treatment Schedule



Note: Negative pregnancy test within 7 days prior to starting chemotherapy regimen (females of child-bearing potential only)

5.3.2 Transplant Preparative Regimen

The conditioning regimen will be one of two options:

Doses:

- **Fractionated total body irradiation (fTBI):** 1320 cGy
- **Cyclophosphamide (Cy)¹:** 50 mg/kg x 2 doses

Day -8	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day +1	Day +2
fTBI	fTBI	fTBI	fTBI	Cy	Cy	Rest	Rest	HSPC and T _{reg} (Orca-T) infusion	Rest	T _{con} infusion, then donor CD19/CD22-CAR T infusion

¹ Cyclophosphamide is administered per institutional guidelines.

Doses:

- **Thiotepa:** 5 mg/kg x 2 doses
- **Busulfan:** 3.2 mg/kg x 3 doses
- **Fludarabine:** 60 mg/m², 50 mg/m² x 2 doses

Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day +1	Day +2
Bu/Flu	Bu/Flu	Bu/Flu	TTP	TTP	Rest*	HSPC and T _{reg} (Orca-T) infusion	Rest	T _{con} infusion, then donor CD19/CD22-CAR T infusion

* Two days of rest are allowed at the discretion of the treating physician.

Routine anti-emetic prophylaxis and treatment should be employed. Corticosteroids may not be used (except for physiologic replacement) or after discussing with the protocol PI.

Subjects must meet all the following criteria before initiating the conditioning chemotherapy regimen:

- ✓ Must be at least 14 days, or 5 half-lives, since the last dose of standard chemotherapy, or 7 days, or 5 half-lives, since the last dose of ALL maintenance therapy; with the exception of corticosteroids, which can continue until starting the conditioning chemotherapy regimen on Day -5
- ✓ No evidence of uncontrolled infection,
- ✓ No clinically significant cardiac dysfunction,
- ✓ Serum creatinine must be < 2 x ULN,

- ✓ No acute neurological toxicity > Grade 1 (with the exception of peripheral sensory neuropathy)
- ✓ Repeat disease evaluation, at the discretion of the investigator, if the subject received any antineoplastic therapy after enrollment if greater than 28 days has elapsed between date of enrollment and start of conditioning lymphodepletion chemotherapy regimen, or if investigator believes disease may have significantly advanced since enrollment.

5.4 Orca-T infusion

5.4.1.1 Premedications

Premedication with acetaminophen (e.g., 500–1000 mg) and diphenhydramine (e.g., 25–50 mg) should be administered prior to administration of each component of Orca-T, unless contraindicated. Acetaminophen may be omitted if necessary or required by institutional guidelines.

5.5 Donor CD19/CD22-CAR T cell Infusion

5.5.1 Cell Infusion Criteria

Given the possibility that a subject could develop CRS or neurotoxicity after discharge from the hospital, subjects will be asked to remain within commuting distance to the clinic for the first 28 days post CD19/CD22 CAR T cell infusion. Subjects and their family members/caregivers will be educated on potential symptoms such as fever, dyspnea, confusion, aphasia, dysphasia, somnolence, encephalopathy, ataxia, or tremor. If subjects develop these symptoms, they will be instructed to immediately contact the investigator or seek immediate medical attention.

Subjects must meet the following criteria in order for cells to be infused (based on labs obtained within 24 hours of cell infusion):

- ✓ CD19/CD22-CAR T cells must have met release criteria
- ✓ No evidence of hemodynamic instability
- ✓ No new requirement for continuous (>24 hours) supplemental oxygen therapy
- ✓ No symptoms concerning for new condition that in the opinion of the PI may pose an unacceptable risk to the subject
- ✓ No evidence of clinically significant cardiac dysfunction, uncontrolled, significant tumor lysis syndrome, serum creatinine > 2 x ULN, and no acute neurological toxicity > Grade 1 (with the exception of peripheral sensory neuropathy).

Corticosteroid use may not exceed ≥ 5 mg/day of prednisone or equivalent for 3 days prior CAR-T infusion.

Other immune suppression medication use is not allowed up to, and during the day of CAR-T cell infusion unless specific exception is granted by the study PI.

Premedications

All subjects will receive levetiracetam (Keppra) 500 mg orally twice a day beginning the day before donor CD19/CD22 CAR T cell infusion. At the onset of $\geq$ Grade 2 neurotoxicity, levetiracetam (Keppra) should be increased from a dose of 500 mg (PO or IV) BID to a dose of 1000mg (PO or IV) BID. Levetiracetam (Keppra) should continue through day 28 once neurotoxicity develops. This agent will be provided by the institution's Pharmacy Department. Please refer to the package insert for complete pharmaceutical information on this product.

Subjects at high risk of TLS, per investigator's discretion, will receive allopurinol 300 mg orally once daily beginning the day prior to CD19/CD22 CAR T cell infusion.

Subjects will receive the following medications 30-120 minutes prior to cell infusion:

- Diphenhydramine 25-50 mg/dose PO or IV;
- Acetaminophen 500-1000 mg/dose PO or IV.

5.6 Treatment Dose

The administration of the CD3⁺ T_{con} dose will occur as the first infusion followed by the CD19/CD22-CAR T cell infusion. The CD19/CD22-CAR T cell infusion should occur no sooner than **30 minutes** after the T_{con} infusion.

CAR-T infusions are performed per institutional guidelines.

At least 3 patients will be treated at each dose level (Table 5 CD19/CD22-CAR T cell Dose Schema. There will be 4 weeks staggering between each patient within a given cohort. The 4-week staggering will be from the time of CD19/CD22-CAR T cell infusion to the time of Orca-T infusion for the next patient. All patients treated at the preceding dose level will be observed a minimum of 6 weeks post T cell infusion before dose escalation occurs.

5.6.1 Confirmation of Engraftment

Engraftment will be confirmed prior to dosing additional subjects. Based on clinical experience, subjects who do not achieve engraftment by Day 42, will be evaluated for subsequent therapy with a second allogeneic hematopoietic cell transplant.

All subjects will be followed even if they do not engraft.

5.7 Potential Considerations for CAR T cell Infusion

5.7.1 Potential Impact of Tacrolimus on CAR T cell Activity

Donor CD19/CD22 CAR T cells will be administered on Day +2 following allo-HCT, and GVHD prophylaxis with tacrolimus will be initiated on Day +3. Although tacrolimus is important in mitigating GVHD, its impact on the CAR T cell phenotype and function is limited. One published study found that tacrolimus blunted the cytotoxicity of CD19-targeted CAR T cells in an in vitro model¹.

To mitigate potential deleterious impacts of tacrolimus on donor CD19/CD22 CAR T cell proliferation, expansion, and activity, we will employ the following strategies.

First, we will target a lower than usual level of tacrolimus in the clinical study. We will target a tacrolimus range of level 5 to 7 as opposed to a level of 5 to 15. It should take approximately 2.5 days for tacrolimus to reach steady state in the allo-HCT recipients.

Second, as noted above, the donor CD19/CD22 CAR T cells will be infused one day prior to the initiation of tacrolimus.

We anticipate that this approach will enable the donor CD19/CD22 CAR T cells to expand prior to potential blunting of their expansion by tacrolimus.

5.8 Dose Modifications and Dose-limiting Toxicities

Adverse event documentation and reporting is described in **Section 7**.

Table 5 CD19/CD22-CAR T cell Dose Schema

Dose Level	CD3⁺ T_{con} Dose	CAR T cell Dose
-1	3x10 ⁶ cells/kg	0.5x10 ⁶ CAR+ cells/kg
1	3x10 ⁶ cells/kg	1x10 ⁶ CAR+ cells/kg
2	3x10 ⁶ cells/kg	2x10 ⁶ CAR+ cells/kg
3	3x10 ⁶ cells/kg	3x10 ⁶ CAR+ cells/kg

The doses in Table 5 will be used for the donor derived CD19/CD22CAR T cells ($\pm 20\%$). We will employ a Bayesian dose escalation design.

At least 3 patients will be treated at the currently selected dose level. There will be 4 weeks staggering between each patient within a given cohort. The 4-week staggering will be from the time of CD19/CD22-CAR T cell infusion to the time of Orca-T infusion for the next patient. All patients treated at the preceding dose level will be observed a minimum of 6 weeks post T cell infusion before dose escalation occurs. Dosing of the donor CD19/CD22-CAR T cells will proceed as outlined in the statistical plan in **Section 10**.

Once the dose escalation cohorts are complete with no DLTs, that the dose expansion portion of the protocol does not require a stagger.

DLT defined in **Section 10.1.1:**

- Acute GVHD Grade III to IV (greater than 15% of the patients)
- Grade 3 ICANS that does not improve to Grade 2 within 72 hours
- Grade 4 non-hematologic toxicity of any duration that are at least possibly related to the administration of CAR T cell infusion and protocol treatment
- Grade 3 toxicity of any duration involving vital organs (cardiac, pulmonary) that are at least possibly related to the administration of CAR T cell infusion and protocol treatment
- Other Grade 3 toxicity lasting > 72 hour that is possibly related to the administration of your CAR T cell infusion and protocol treatment. **Exceptions may be made for Grade 3 abnormal hepatic or renal function tests that improve to grade 2 or less within 7 days.**
- Grade 3 CRS that does not improve to a Grade 2 or less in 5 days following adequate therapy
- Grade 3 ICANS that does not improve to a Grade 2 or less in 5 days following adequate therapy
- Any Grade 4 CRS or ICANS
- In the standard of care allogeneic hematopoietic cell transplant, there is an anticipated 15 to 20 percent incidence of GVHD 3 or 4 by Day100. Hence, we propose the following DLTs pertaining to Grade 3 to 4 acute GVHD. If we see 2 of 4, 3 of 10, or 4 of 15 cases of Grade 3 or 4 GVHD, then we will consider it as a DLT. **This event will trigger a pause in the study as well as a DSMC and FDA review.**
- Death (except due to disease progression)

The following expected events will be considered **DLT** exceptions:

- Grade 3 or 4 infusion-related toxicities that are reversible to $\leq$ Grade 2 within 8 hours
- Grade 3 abnormal laboratory values without clinical consequence or outcome will not be recorded as adverse events, unless deemed serious
- Grade 3 fever, Grade 4 fever responsive to antipyretic treatment or febrile neutropenia for ≤ 2 weeks can be excluded as DLTs
- Grade 3 or 4 tumor lysis syndrome (TLS) for ≤ 2 weeks
- Grade 3 hypotension (without other CRS symptoms) requiring a single vasopressor for support that resolves to < Grade 3 in ≤ 72 hours can be excluded as a DLT

Rationale for DLT Definition

Grade 3-4 toxicities, including but not limited to hematologic, gastrointestinal, infectious, pulmonary, renal, and cardiac may occur after standard allogeneic hematopoietic cell transplantation and therefore will not be considered DLTs throughout the course of this study. Therefore, we have chosen the **DLT** definition to include Grade 3-4 GVHD.

MTD is defined as the dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. Refer to Section 10.1.4 for details.

Stopping Rules for Delayed Toxicity

Dose escalation will proceed based upon DLT experienced within the treatment and observation periods as described above. Toxicities observed after the minimum of 6 weeks that are possibly, probably, or definitely related to treatment with donor CD19/CD22-CAR cells will be collected and evaluated by the investigators and reported to the Stanford IRB, Oversight Committee, Investigational Drug Committee, and the FDA.

Stopping rules were developed based on the following events. These events, which will lead to a pause in the study, will be assessed for patients within a given dose level. If any of the following toxicities are noted, then accrual will be held pending analysis and will be restarted only after approval from the appropriate Stanford Committees:

- *Episode of Grade 3 or 4 ICANS greater than 7 days:* Monitor the number of patients who experience this episode when either 3 or 6 patients have been treated at a dose level. Pause the study if the number is at least 2 out of 3 or at least 3 out of 6.
- *Graft Failure by Day 42:* Monitor the number of patients who experience graft failure when either 3, 6, or 9 patients have been treated at a dose level. Pause the study if the number is at least 2 out of 3, at least 3 out of 6, or at least 4 out of 9.
- Any death attributable to the product occurring at any dose level within the 6-week evaluation window should trigger a pause in further enrollment until review by the appropriate Stanford committees and FDA.

5.9 Concomitant Medications, Procedures, and Supportive Care Guidelines

5.9.1 Concomitant Therapy

All concomitant medications, blood products, procedures, radiotherapy, or other treatments with therapeutic intent administered to recipients will be recorded from the beginning of the conditioning regimen through the 6-week safety reporting period. Any concomitant therapy given for a study protocol-related adverse event should be recorded from the time of informed consent. Any treatment for adverse events of special interest (AESI), GVHD of any type, grade $\geq$ 2 infection, or PTLD will be recorded all times. Initial treatment for disease recurrence/relapse must also be recorded.

5.9.2 Prohibited Concomitant Medications or Procedures

Subjects may not receive other investigational therapies (except as allowed by the Principal Director, immunosuppressive medications, radiotherapy, or systemic antineoplastic therapy during the study except as explicitly allowed per protocol.

5.9.3 Supportive Care Guidelines

5.9.3.1 GVHD Prophylaxis

All recipients will receive single-agent GVHD prophylaxis consisting of single-agent tacrolimus. GVHD prophylaxis should begin on the day following donor CD19/CD22-CAR T cells infusion (typically Day +2), starting 12-18 hours after the start of the donor CD19/CD22-CAR T cells infusion.

Tacrolimus should be initiated at 0.015 mg/kg/day for a target range level of 5 to 7. If a trough level of <5 ng/mL is noted prior to the start of tapering of GVHD prophylaxis, tacrolimus dosing should be increased to target the recommended trough level unless clinically contraindicated due to toxicity or after discussion with the PI and/or designee. Per os (PO) administration is permissible when the patient can tolerate food; tacrolimus dosing may be initiated via PO route if tolerated.

5.9.3.2 Tapering of GVHD Prophylaxis

Tacrolimus taper should be initiated at a minimum of 90 days post-HSCT if there is no evidence of active GVHD, with a suggested taper of the prophylaxis regimen by approximately 20% of the dose per month.

5.9.3.3 Modifications to GVHD Prophylaxis

Apart from dose modifications, all modifications to the GVHD prophylaxis regimens must be discussed with the Principal Investigator and/or designee, including in cases of intolerance. The reason(s) for modification will be recorded in the CRF.

5.9.3.4 Antimicrobial Prophylaxis

All patients will receive prophylaxis against bacterial, fungal, and viral infections during the peri-transplant period according to institutional practices.

Recipients and donors with CMV negative statuses do not require CMV testing.

Of note, all CMV-seropositive recipients should receive letermovir prophylaxis beginning between day 0 and day +28; therapy should continue through day +100. Letermovir may be omitted for CMV-seropositive recipients only if contraindicated (e.g., known hypersensitivity to letermovir) with PI or PI designee approval.

5.10 Treatment Duration

Recipient's active participation and short term follow up in this study is expected to be 1 to 2 years, with a total of 15 years of long term follow up after the last subject completes study therapy due to ongoing monitoring required following CAR T cell therapy.

5.11 Criteria for Removing Subjects from Study Intervention and/or Study

Subjects are free to withdraw consent and discontinue participation in a study at any time without prejudice to further treatment. The investigator may discontinue a subject from treatment intervention or the entire study for medical or administrative reasons. The following reasons may lead to discontinuation from the study intervention or the entire study.

- There is need for any treatment not allowed by the protocol
- Disease recurrence or progression
- Toxicity precluding further study treatment
- Withdrawal of consent
- Study non-compliance
- Patient becomes pregnant
- Investigator discretion

If the subject elects to withdraw consent from further treatment with the study intervention, or is withdrawn by the investigator, it will be confirmed and documented if the subject will consent to continue to be followed per protocol, if applicable. The rationale for the investigator's decision to discontinue treatment will be clearly documented.

5.12 Duration of Follow-Up

After completion of treatment (cell infusions), subjects will be followed for a total of 15 years after completion or removal from study or until death, whichever occurs first. If a subject is removed from study due to unacceptable adverse event(s) or toxicity, that subject will be followed and safety data collected for at least **30** days or until resolution or stabilization of the adverse event, whichever is longest, to the extent possible. Subjects that personally withdraw will be requested to attend a 30-day follow-up visit (or longer), or otherwise receive a follow-up contact, unless specifically countermanded by the subject.

5.12.1 Gene Therapy Follow Up Assessments

5.12.1.1 Blood Samples for RCL

RCL blood samples will be collected from subjects returning to clinic or collected by a local physician, clinical or health care agency, and shipped to Indiana University Gene Therapy Testing Laboratory (IU GTTL) according to CCT SOPs, and at the time point specified. If any post-treatment samples are positive, further analysis of the RCL and more extensive subject follow-up will be undertaken, in consultation with the FDA.

If a subject dies or develops neoplasms during this follow up, efforts will be made to assay a biopsy sample for RCR.

5.12.1.2 Clinical Follow Up post Gene Therapy

In compliance with OSP and FDA's ***Guidance for Industry: Long Term Follow-Up After Administration of Human Gene Therapy Products*** (January 2020), subjects who have received at least one dose of a genetically engineered cellular therapy will be evaluated for long term safety and occurrence of adverse events according to the requirements established by FDA. Baseline data from the study participation (i.e. medical history, physical exam, clinical laboratory results) will be used to evaluate changes over time when compared to follow up data

Given the length of follow-up required post gene therapy infusions, it is possible that patients relocate or those who traveled from long-distances to receive study treatment are no longer able to travel to the study site for follow-up. For patients who are not able to travel to the study site for clinical visits, the history, physical exam and standard laboratory tests can be performed by the local medical provider. Additionally, such patients followed elsewhere will be contacted by the coordinator of this study at Stanford to report on targeted concomitant medications and targeted adverse events as outline below.

Patients are asked to keep the investigator and study coordinator abreast of changes in contact information.

Clinical Evaluations: Year 1-5 as follows:

o Targeted Physical Exam and History –

Gene Therapy annual evaluations should specifically document survival, progression status, any new malignancies, new incidence or exacerbation of a pre-existing neurologic disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, new incidence of a hematologic disorder, new incidence of infection (potentially product related); and other factors that may be relevant to the feasibility and scientific

value of the long-term follow up observations (i.e. exposure to other cancer causing agents).

- o Targeted Adverse/Serious Adverse Event reporting including neurological, hematological, infections, autoimmune disorders, and secondary malignancies
- o Targeted concomitant medication documentation including immunosuppressive drugs, anti-infectives, and vaccinations.

Clinical Evaluations: Year 6-15

Once a subject has completed 5 years of follow up without evidence of delayed adverse events secondary to genetically engineered cellular therapy product, the following evaluation will be conducted annually for the subsequent 10 years ($\pm$ 2 months):

The subject will be contacted (in clinic or via phone or written questionnaire) annually to evaluate for survival and development of delayed adverse events (See questionnaire in Appendix F).

- o If vector modified cells were detected in the blood during the previous visit, then blood for persistence of vector modified cells will be collected and tested until negative.
- o Subjects are requested to inform the study research team of any changes to e-mail (if consented to use e-mail), phone, and address. E-mails will be sent to subjects via the Secure Email to ensure securely and confidentially over an SSL/encrypted connection.
- o Distribution and collection of questionnaires

A draft letter to subject's primary doctor/local medical care provider is provided in Appendix H. This letter should be sent to the local health care provider via mail, fax or e-mail at the time the subject is referred back to the care of the local physician or with any change in primary doctors. E-mails will be sent to subjects via the Secure Email to ensure securely and confidentially over an SSL/encrypted connection.

- o Subjects will be sent a request for information (Appendix F) and questionnaire annually.
 - § If there is no response within 1 month, the study coordinator will follow up with a telephone call to request the information. The questionnaire may be completed with the subject responses over the phone at this time.
 - § If there is no response to the telephone call, the letter and questionnaire (Appendix F) should be sent to the subject via FedEx, signature required.
 - § Each attempt to contact the subject and outcome must be documented in the medical record.

5.13 Study Completion or Discontinuation

The study will be complete when the last study data, including all follow-up data, has been collected, and analysis is complete. This is expected to occur when the last subject completes the 15-year follow-up period or removal from the study or until death, whichever occurs first.

The study will be complete when the last study data, including all follow-up, is expected to be collected. In addition, follow up for gene therapy according to the U.S. Food and Drug

Administration (FDA) ***Guidance for Industry: Gene Therapy Clinical Trials – Observing Participants for Delayed Adverse Events*** will be conducted on this study or a long-term follow up study for subjects participating in genetic research for the required 15 years, for a total of 18 - 19 years until study completion.

Subjects will no longer be followed, or subject data collected once the study is complete per this section.

Conditions may be discovered during the conduct of the study by the investigator, regulatory authorities, or other oversight bodies that indicate that the study should be terminated prematurely. The reasons that may warrant termination include, but are not limited to:

- The study is determined to be non-feasible, including inadequate accrual
- Determination of unexpected, significant, and/or unacceptable risk to participants
- Demonstration of efficacy that would warrant termination (sponsor or investigator decision)
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination of futility
- Business decision by the Principal Investigator, institution, or funding source, etc

Written notification will be provided by the investigator to the IND sponsor, the funding entity, and regulatory authorities as appropriate, including the IRB of record.

6 STUDY PROCEDURES AND ASSESSMENTS

6.1 Schedule of Events

Schedule of Events: DONOR

	Screening	Apheresis	Mobilization	Apheresis
Study Day (relative to HSPC/T _{reg} infusion = Day 0)	Baseline Must be completed within 45 days of apheresis	-9/-8 ¹	-6 to -2/-1 ²	-2/-1 ²
Window		± 1 day	± 1 day	± 1 day
Informed consent	X			
Inclusion/Exclusion Criteria	X			
Demographics	X			
Medical history	X			
Physical exam	X			
Vital signs	X	X		X
CBC with differential	X	X	Daily x4 or per institutional practice	X
Clinical Chemistry	X			
Coagulation	X			
Donor Infection Disease Markers	X			
Evaluation for COVID-19 Risk*	X			
Serum or urine β-HCG (if WOCBP4)	X			
ABO and Rh typing	X			
G-CSF mobilization			Daily x5	
Apheresis		X		X
Correlative studies		X		

Footnotes:

*Evaluation of COVID-19 risk will be performed according to the most current version of the BMT-CT COVID SOP.

1. In the event a second day of unmobilized collection is needed.
2. In the event a second day of mobilized collection is needed.

Schedule of Events: RECIPIENT

	Pre-Transplant Monitoring Period		Cell Infusions			Post-Transplant Assessment Period						Long Term Follow-Up		
Procedure	Screening	MAC	HSPC T _{reg} (Orca-T) Infusion	Prior to cell infusions (< 24 h)	T _{con} Infusion & Donor CD19/CD22 CAR T cell Infusion	Supportive Care/Continuation of Inpatient Monitoring ¹¹								
Study Day (vs. 1 st allo-HCT infusion)	Must be completed within 60 days of conditioning regimen	Days -8 to -2 or Days -6 to -2	Day 0	Day 1	Day 2	Daily, Day 1 to Day 21	Twice weekly, Day 22 to Day 29	Day 30	Day 42	Day 60	Month 3, 6, 12	Every 6 Months 1 to 5 Years	Annually, Year 6 to 15	At Disease Relapse
Window						± 2 d	± 4 d	± 4 d	± 4 d	± 1 week	± 2 weeks	± 2 mo.	± 3 mo.	
Medical and Disease History	X													
Prior Therapies	X													
Physical Exam	X		X	X	X	X		X		X	X			
Vital signs	X	X	X	X	X	X	X	X		X	X			
Neurologic exam	X			X		X		X		X	X			
Height (screening only) and weight	X	X	X	X						X	X			
Karnofsky Score or ECOG	X		X					X		X	X	X		

	Pre-Transplant Monitoring Period		Cell Infusions			Post-Transplant Assessment Period						Long Term Follow-Up		
Procedure	Screening	MAC	HSPC _{Treg} (Orca-T) Infusion	Prior to cell infusions (< 24 h)	T _{con} Infusion & Donor CD19/CD22 CAR T cell Infusion	Supportive Care/Continuation of Inpatient Monitoring ¹¹								
Study Day (vs. 1 st allo-HCT infusion)	Must be completed within 60 days of conditioning regimen	Days -8 to -2 or Days -6 to -2	Day 0	Day 1	Day 2	Daily, Day 1 to Day 21	Twice weekly, Day 22 to Day 29	Day 30	Day 42	Day 60	Month 3, 6, 12	Every 6 Months 1 to 5 Years	Annually, Year 6 to 15	At Disease Relapse
Window						± 2 d	± 4 d	± 4 d	± 4 d	± 1 week	± 2 weeks	± 2 mo.	± 3 mo.	
Evaluation for COVID-19 Risk ¹	X													
	Labs													
• Coagulation	X			X		X ⁷	X	X		X				
• CBC with diff	X	X	X	X		X	X	X		X	X	X		
• Chemistries	X	X	X	X		X	X	X		X	X			
• Phosphorus	X		X					X						
• Magnesium	X	X	X	X		X		X						
• CRP	X	X	X	X		X	X	X		X				

	Pre-Transplant Monitoring Period		Cell Infusions			Post-Transplant Assessment Period						Long Term Follow-Up		
Procedure	Screening	MAC	HSPC T _{reg} (Orca-T) Infusion	Prior to cell infusions (< 24 h)	T _{con} Infusion & Donor CD19/CD22 CAR T cell Infusion	Supportive Care/Continuation of Inpatient Monitoring ¹¹								
Study Day (vs. 1 st allo-HCT infusion)	Must be completed within 60 days of conditioning regimen	Days -8 to -2 or Days -6 to -2	Day 0	Day 1	Day 2	Daily, Day 1 to Day 21	Twice weekly, Day 22 to Day 29	Day 30	Day 42	Day 60	Month 3, 6, 12	Every 6 Months 1 to 5 Years	Annually, Year 6 to 15	At Disease Relapse
Window						± 2 d	± 4 d	± 4 d	± 4 d	± 1 week	± 2 weeks	± 2 mo.	± 3 mo.	
• Ferritin	X	X				X	X							
• LDH		X			X	X	X							
• Uric acid	X	X	X											
• TSH	X				X			X			X			
• Chimerism								X		X	X			
• Recipient Infectious Disease Markers	X													
• PCR-based testing for SARS-CoV-2	X													
• Serum Ig Levels (IgM, IgA, IgG)	X		X					X		X				X
• Urine analysis	X			X										
• Serum or urine β - HCG (if WOCBP)	X													

	Pre-Transplant Monitoring Period		Cell Infusions			Post-Transplant Assessment Period						Long Term Follow-Up		
Procedure	Screening	MAC	HSPC T _{reg} (Orca-T) Infusion	Prior to cell infusions (< 24 h)	T _{con} Infusion & Donor CD19/CD22 CAR T cell Infusion	Supportive Care/Continuation of Inpatient Monitoring ¹¹								
Study Day (vs. 1 st allo-HCT infusion)	Must be completed within 60 days of conditioning regimen	Days -8 to -2 or Days -6 to -2	Day 0	Day 1	Day 2	Daily, Day 1 to Day 21	Twice weekly, Day 22 to Day 29	Day 30	Day 42	Day 60	Month 3, 6, 12	Every 6 Months 1 to 5 Years	Annually, Year 6 to 15	At Disease Relapse
Window						± 2 d	± 4 d	± 4 d	± 4 d	± 1 week	±2 weeks	±2 mo.	±3 mo.	
• ABO and Rh Typing	X													
• HLA Typing and anti-donor HLA antibody testing ²	X													
• RCL blood sample				X ⁸							X			
Electrocardiogram, 12-lead	X													
ECHO, MUGA or cardiac MRI	X													
DLCO Pulmonary Function Test	X													
EBV/CMV NAT						Weekly from Day 7 through Day 60 ⁶					X	X		
Correlative Research Studies		Correlative samples outlined in Section 8												

	Pre-Transplant Monitoring Period		Cell Infusions			Post-Transplant Assessment Period						Long Term Follow-Up		
Procedure	Screening	MAC	HSPC _{Treg} (Orca-T) Infusion	Prior to cell infusions (< 24 h)	T _{con} Infusion & Donor CD19/CD22 CAR T cell Infusion	Supportive Care/Continuation of Inpatient Monitoring ¹¹								
Study Day (vs. 1 st allo-HCT infusion)	Must be completed within 60 days of conditioning regimen	Days -8 to -2 or Days -6 to -2	Day 0	Day 1	Day 2	Daily, Day 1 to Day 21	Twice weekly, Day 22 to Day 29	Day 30	Day 42	Day 60	Month 3, 6, 12	Every 6 Months 1 to 5 Years	Annually, Year 6 to 15	At Disease Relapse
Window						± 2 d	± 4 d	± 4 d	± 4 d	± 1 week	±2 weeks	±2 mo.	±3 mo.	
Disease evaluation														
• Brain MRI (or other appropriate imaging only, if necessary, based on the site of disease) ³	X							X		X ³	X ³			
• Lumbar puncture ⁴	X ⁴							X ⁴			X ⁴			
• Bone marrow aspirate with MRD	X								X		X			
• Engraftment assessment									X					
• MRD blood sample ¹²	X							X		X	X	X		
• T and B cell lymphocyte subsets								X		X	X	X		

	Pre-Transplant Monitoring Period		Cell Infusions			Post-Transplant Assessment Period						Long Term Follow-Up		
Procedure	Screening	MAC	HSPC T _{reg} (Orca-T) Infusion	Prior to cell infusions (< 24 h)	T _{con} Infusion & Donor CD19/CD22 CAR T cell Infusion	Supportive Care/Continuation of Inpatient Monitoring ¹¹								
Study Day (vs. 1 st allo-HCT infusion)	Must be completed within 60 days of conditioning regimen	Days -8 to -2 or Days -6 to -2	Day 0	Day 1	Day 2	Daily, Day 1 to Day 21	Twice weekly, Day 22 to Day 29	Day 30	Day 42	Day 60	Month 3, 6, 12	Every 6 Months 1 to 5 Years	Annually, Year 6 to 15	At Disease Relapse
Window						± 2 d	± 4 d	± 4 d	± 4 d	± 1 week	± 2 weeks	± 2 mo.	± 3 mo.	
	Treatment Regimen													
• Continuing Regimen ⁵		X												
• Tacrolimus ¹⁰						X ¹⁰	X	X		X				
• Levetiracetam (Keppra) ¹³				X	X	X	X							
• Letermovir				All CMV-seropositive recipients should receive letermovir prophylaxis beginning between day 0 and day +28; therapy should continue through day +100. Other patients may receive letermovir per institutional practice.										
• HSPC T _{reg} (Orca-T) Infusion			X											
• Donor CD19/CD22-CAR T cell Infusion					X									
• T _{con} Infusion					X									
Response Evaluation								X		X	X	X	X	X
GVHD Assessment						X ⁹	X	X		X	X*			

	Pre-Transplant Monitoring Period		Cell Infusions			Post-Transplant Assessment Period						Long Term Follow-Up		
Procedure	Screening	MAC	HSPC T _{reg} (Orca-T) Infusion	Prior to cell infusions (< 24 h)	T _{con} Infusion & Donor CD19/CD22 CAR T cell Infusion	Supportive Care/Continuation of Inpatient Monitoring ¹¹								
Study Day (vs. 1 st allo-HCT infusion)	Must be completed within 60 days of conditioning regimen	Days -8 to -2 or Days -6 to -2	Day 0	Day 1	Day 2	Daily, Day 1 to Day 21	Twice weekly, Day 22 to Day 29	Day 30	Day 42	Day 60	Month 3, 6, 12	Every 6 Months 1 to 5 Years	Annually, Year 6 to 15	At Disease Relapse
Window						± 2 d	± 4 d	± 4 d	± 4 d	± 1 week	± 2 weeks	± 2 mo.	± 3 mo.	
Adverse Events		X	X	X	X	X	X	X		X	X			X
Concomitant Medications	X	X	X	X	X	X	X	X		X	X			X

Footnotes:

- 1) Evaluation of COVID-19 risk will be performed according to the most current version of the BMT-CT COVID SOP
- 2) HLA typing can occur prior to the 60-day screening window and will be accepted for eligibility and enrollment procedures.
- 3) Only if abnormal at Day 30.
- 4) Only if prior CNS involvement of ALL per investigator discretion.
- 5) During the TBI regimen, in the outpatient setting, daily labs and vitals will not be collected. Labs and vitals will be collected at least 2x per week.
- 6) CMV/EBV monitoring will start on Day 7 and proceed weekly until Day 60.
- 7) Coagulation performed 2x/weekly during the Day 1 to Day 21 timeframe.
- 8) Day 1 RCL – any time prior to infusion is appropriate for baseline.
- 9) GVHD assessment will be performed weekly during the Day 1 to Day 21 timeframe.
- 10) Tacrolimus starts on Day +3 (starts the day after T_{con} infusion but not sooner than 12 hours post infusion) and will be administered per protocol guidelines.
- 11) Based on the subject status and progress during the Supportive Care/Continuation of Inpatient Monitoring period, the subject may be transitioned to outpatient supportive care and continuation of monitoring per investigator discretion.
- 12) MRD blood sample are optional at all timepoints.
- 13) Keppra is administered per institutional guidelines.

6.2 Description of Procedures and Assessments

6.2.1 Safety and Other Assessments

The following clinical and safety assessments will be performed according to the Study Procedures and Assessments.

1. History and Physical Examination

Standard medical history is acceptable. Following screening, updates to medical history should be recorded. A complete physical examination as well as a cancer-specific physical exam will be performed at screening. The exam will include general appearance of the subject, height and weight, examination of the skin, eyes and ears, nose, throat, lungs, heart, abdomen, extremities, musculoskeletal system, and a thorough nervous system evaluation.

Physical exams at subsequent applicable visits will be targeted based on the investigator's clinical observations and symptomatology.

2. Vital Signs (VS)

Vital signs, including blood pressure, heart rate, oxygen saturation and temperature will be monitored per institutional guidelines for each component of CD34⁺ HSPC, T_{reg}, T_{con}, and CD19/CD22-CAR T cell infusions.

- a. Supplemental oxygen will be available at the bedside.
- b. If an allergic or other acute reaction occurs, studies appropriate for investigation of a transfusion reaction will be performed (urinalysis, CBC, Coomb's test).
- c. Acute reactions or increased intracranial pressure will be treated according to institutional standards of care, while avoiding steroid administration if possible.

3. Performance Status (PS)

Performance Status Criteria

Karnofsky Status	Karnofsky Grade	ECOG Grade	ECOG Status
Normal, no complaints	100	0	Fully active, able to carry on all pre-disease performance without restriction
Able to carry on normal activities. Minor signs or symptoms of disease	90	1	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
Normal activity with effort	80	1	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
Care for self. Unable to carry on normal activity or to do active work	70	2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
Requires occasional assistance, but able to care for most of his needs	60	2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
Requires considerable assistance and frequent medical care	50	3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
Disabled. Requires special care and assistance	40	3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
Severely disabled. Hospitalization indicated though death nonimminent	30	4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
Very sick. Hospitalization necessary. Active supportive treatment necessary	20	4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
Moribund	10	4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
Dead	0	5	Dead

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4. Targeted Adverse/Serious Adverse Event Reporting

Detailed information about the collection and reporting of adverse and serious adverse events is outlined in **Section 7 Adverse Events And Reporting Procedures**.

5. Targeted Concomitant Medication Documentation

After Day 60, Month 3, 6, 12 ($\pm$ 2 weeks), or until disease relapse (whichever occurs first), targeted concomitant medication collection of gammaglobulins, immunosuppressive drugs, anti-infectives, and vaccinations will be performed.

6. RCL Testing

The US Food and Drug Administration requires monitoring for replication competent lentivirus in all human subjects receiving lentiviral gene therapy. Even if a subject withdraws from other study evaluations, permission to continue RCL testing and annual gene therapy questionnaires will be requested. Blood collection of RCL testing will be performed as described in **Appendix**

F: Monitoring Gene Therapy Trials: Replication Competent Lentivirus (RCL) at the following timepoints:

Subject Testing for RCL PCR

Subject blood samples will be obtained at the following time points and banked for future testing:

1. Prior to cell infusion
2. 3 months($\pm$ 1 month)
3. 6 months ($\pm$ 1 month)
4. 12 months ($\pm$ 2 months)

All Samples are scheduled from the most recent cell infusion, and should proceed even after disease progression or proceeds to alternative therapy; the only exception is if the subject proceeds to stem cell transplant with myeloablative preparative regimen. RCL testing of archived samples will occur if events are considered concerning for long-term new or worsening suspected adverse reaction or for events described in **Section 8**.

7. Primary Endpoint

The first primary endpoint is the safety of the donor CD19/CD22CAR T cells plus Orca-T infusion will be assessed by the incidence of engraftment without Grade III to IV acute GVHD at Day 60.

The incidence and timing of neutrophil engraftment through Day +42

- Neutrophil engraftment is defined as achieving an absolute neutrophil count (ANC) $\geq 500/\text{mm}^3$ for 3 consecutive days, by Day +42. The first of the three days will be designated the day of engraftment.

Primary graft failure will be defined as follows:

- patient alive without recovery of neutrophils (i.e., without achieving an absolute neutrophil count [ANC] $\geq 500/\text{mm}^3$ for 3 consecutive days) at Day +42 without another identifiable cause such as relapsed/persistent disease.

6.2.2 Efficacy Assessments

Participants will be evaluated for disease evaluation using brain MRI, lumbar puncture, bone marrow aspirate with MRD, MRD blood sample, and T and B cell lymphocyte subsets.

Note: It is recommended but not required to measure T and B cell lymphocyte subsets monthly starting at Day 30 to monitor for return of B cells, a possible indicator of loss of CAR T cell persistence.

6.3 Procedures and Assessments Risk Information

6.3.1 Risks of Apheresis

Apheresis is a safe procedure that is routinely performed in healthy adults. Participants will be closely monitored and procedures to minimize risks and prevent side effects are incorporated into all aspects of the protocol. The institutions have broad expertise to adequately manage side effects. The potential risks of apheresis in this trial are as follows:

- a. The most common side effects of apheresis are pain and bruising at IV sites. A central venous catheter may be required. Possible side effects include pain, bleeding, bruising, infection, thrombosis, vascular perforation.
- b. During apheresis, mild side effects from citrate anticoagulant are common and include chills, numbness and tingling ("pins and needles"), anxiety, muscle cramps, and nausea. More serious side effects due to citrate-induced hypocalcemia are uncommon and include low blood pressure, seizures, weakness, and tetany. Citrate reactions rapidly resolve when the collection is slowed down or stopped. Prophylactic IV CaCl_2 and MgSO_4 infusions may be administered to subjects deemed to be at high risk of citrate toxicity. Risks of parenteral calcium and magnesium include extravasation necrosis and cardiovascular effects including bradycardia and blood pressure changes. However, side effects are unlikely given the low rate of infusion and use of large bore catheters for apheresis.
- c. Transient mild thrombocytopenia is common after apheresis, but bleeding is unlikely.
- d. Dilutional anemia occurs during apheresis, but this is unlikely to be clinically significant.
- e. Side effects of blood draws include pain and bruising, lightheadedness, and rarely, fainting.

6.3.2 Toxicities of conditioning regimens and supportive agents

6.3.2.1 Fludarabine

Likely

- Exacerbation of nausea, vomiting, mouth sores, and diarrhea, which are primarily due to the cyclophosphamide.
- Immune suppression

Less likely

- Jaundice and elevations of liver enzymes

Rare but serious

- Effects on the nervous system are rare, but if they occur could include confusion, coma, weakness or numbness, loss of balance, difficulty walking, or loss of vision and could be very serious or lethal.

6.3.2.2 Thiotepea

Likely

- Myelosuppression is the major dose-limiting toxicity, occurring regularly at doses $>405\text{mg/m}^2$. Other non-fatal toxicities have been observed almost exclusively after administration of thiotepea at doses greater than 1000mg/m^2 .
- Increased risk of infection.
- Cutaneous erythema and bronzing are seen in most patients given 900mg/m^2 . Erythema develops 4-14 days after the first dose and may last up to 3 weeks. Bronzing may persist for months.

Less likely

- Nausea, vomiting, diarrhea - occasional, rarely severe

Rare but Serious

- CNS toxicity manifested by mild cognitive dysfunction, disorientation, confusion, irritability, bizarre behavior, is usually not observed at doses <1000mg/m².
- Interstitial pneumonia
- Renal failure
- Transient hepatic transaminase elevations are occasionally seen, but rarely severe.

6.3.2.3 Busulfan

Busulfan is a chemotherapy drug that has the following side effects nausea, vomiting, diarrhea, sores in the mouth and throat, darkening of the skin, seizures and low blood counts. In rare circumstances, busulfan may result in permanent hair thinning or loss. Busulfan can cause liver damage which may be fatal. There is also a risk of the development of a second cancer.

6.3.2.4 Total Body Irradiation (TBI)Likely

- Nausea and vomiting: Virtually all patients will experience nausea and vomiting after irradiation. This can be diminished somewhat with mild anti-emetics. Strong sedatives or phenothiazine derivatives should be avoided just before radiation treatment, as they frequently cause excessive drowsiness and/or symptomatic orthostasis, which prevent delivery of TBI done in the standing position. We will limit the use of dexamethasone for nausea unless discussed with the study PI.
- Myelosuppression is the major dose-limiting toxicity.
- Hyperpigmentation: Most patients will get some degree of hyperpigmentation within 2-3 weeks of transplantation.
- Increased risk of infection
- Mucositis: Most patients will develop moderate to severe mucositis of the oral and GI tracts, which will be managed with aggressive nursing mouth care, analgesics and prophylactic antifungal and antiviral agents. We will limit the use of dexamethasone for mucositis unless discussed with the study PI.
- Late effects: There is the possibility of cataract formation. Although mild cataracts may occur in up to 70% of cases with single dose TBI, we have seen cataracts in < 30% of patients treated with hyperfractionated TBI.
- Sterility is extremely common following total body irradiation and administration of alkylating chemotherapy; the risk increases with the number of years since puberty.
- Diarrhea: Most patients develop some diarrhea in the first week post irradiation. This can be treated symptomatically.

Less likely

- Parotitis: Some patients will experience symptomatic parotitis within the first 24 hours post radiation. This resolves spontaneously over several days.
- Fever: Low-grade fever [greater than 38°C] may occur for 24-hour post irradiation. This can be treated symptomatically.
- Erythema: This may occur in patients within 24 hours and resolves in 2-3 days.
- Hypothyroidism has been reported in small numbers of adult patients following TBI plus alkylating chemotherapy, and this will be routinely monitored post-transplant with hormonal replacement as indicated.

Rare but serious

- High doses of radiation in combination with high dose chemotherapy may contribute to damage to vital organs such as the kidneys, lung or the liver.
- There is a possibility that secondary malignancies may develop, particularly due to the combined effects of radiation and an alkylating agent [cyclophosphamide]. The age-adjusted incidence of secondary cancer in transplant patients after radiation and chemotherapy has recently been estimated to be 6.7 times higher than that of first cancers in the general population; and most of these were non-Hodgkin's lymphomas.

6.3.2.5 Cyclophosphamide

Cyclophosphamide is a chemotherapy drug that has the following side effects nausea, vomiting, diarrhea, sores in the mouth and throat, skin changes, loss of appetite, irritation and bleeding of the bladder lining and lowered blood counts. Cyclophosphamide may also damage the heart which in some cases can be fatal. There is also a risk of the development of a second cancer.

6.3.2.6 G-CSF (Neupogen)

Side effects of G-CSF are generally mild, include bone pain, headaches, body aches, fatigue, edema, and nausea and are managed with supportive care. Pleuro- or pericarditis are seen rarely and are managed by cessation of the medication and corticosteroids if necessary.

6.3.2.7 TacrolimusLikely

- Headache and/or dizziness
- Uncontrollable shaking of a part of the body
- Diarrhea and/or constipation
- Nausea and/or vomiting
- Heartburn
- Stomach pain
- Loss of appetite
- Difficulty falling asleep and/or staying asleep

Less likely

- Damage to organs (heart, lungs, brain, others) which may cause changes in thinking, confusion, memory loss or shortness of breath
- Allergic reaction which may cause rash, low blood pressure, wheezing, shortness of breath, swelling of the face or throat
- Change in the heart rhythm, abnormal heartbeat, or heart stops beating
- Heart attack or failure which may cause chest pain, swelling of ankles, and tiredness
- A tear or a hole in the bowels which may cause belly pain or that may require surgery
- A new cancer resulting from treatment of earlier cancer
- Brain damage, which may cause headache, seizure, blindness

Rare but serious

- Decreased urination
- Pain or burning on urination
- Swelling of the arms, hands, feet, ankles, or lower legs
- Weight gain
- Unusual bleeding or bruising
- Seizures
- Coma (loss of consciousness for a period of time)

6.3.2.8 LetermovirLikely

- Bloating or swelling of the face, arms, hands, lower legs, or feet
- rapid weight gain
- tingling of the hands or feet
- unusual weight gain or loss
- Cough
- diarrhea
- headache
- nausea
- stomach pain
- unusual tiredness or weakness
- vomiting

Less likely

- Dizziness
- fainting
- fast, pounding, or irregular heartbeat or pulse

6.3.3 Cellular Infusions**6.3.3.1 Orca-T**

Orca-T is developed using an experimental process, and its administration for allogeneic transplant is experimental.

6.3.3.1.1 Risks Associated with Orca-T

As of October 29, 2021, 109 subjects have been treated with Orca-T. Because of limited prior experience, the risks and side effects of Orca-T are still being identified. If important findings regarding the risks and side effects of Orca-T are discovered during the clinical trial, this information will be provided the clinical and research personnel. The following are possible risks:

- Manufacturing failure. The production of Orca-T is an experimental process. It is possible that an Orca-T may not be able to be created. If that happens, the available cells from the donor could be used to create a graft for you, but you may not be able to be enrolled in this

research study. Alternative methods may be necessary to secure sufficient cells from your donor, for example a bone marrow harvest.

- Allergy or hypersensitivity. The manufacturing of Orca-T includes iron dextran, chemical products derived from cyanine dyes, and proteins derived from mice, cows, algae and a type of bacterium called *Streptomyces avidinii*. Some people have severe allergic reactions to these products, including anaphylaxis or other potentially fatal reactions. You should not receive Orca-T if you have a known allergy or hypersensitivity to any of the above components.
- Infusion reactions. Infusion reactions have sometimes been reported when cells are infused into patients for allogeneic transplant. It is not known specifically what cells are responsible for such reactions, but they may include the cell types used in Orca-T. You will be given medications to lessen the risk of developing an infusion reaction. In addition, you will be monitored closely for signs of infusion reactions after being given Orca-T components, and appropriate treatment initiated as soon as possible.
- Worsening of transplant-related outcomes. Orca-T is designed to improve outcomes in transplantation by reducing the risk of complications like graft versus host disease, disease relapse and infection, while improving overall survival. However, until proven, it is possible that Orca-T may actually worsen one or more of these outcomes. The research team will be monitoring these events in all patients while the study is ongoing.
- One case of donor hematopoietic graft loss has been noted in a clinical trial patient who had autologous recovery of their own bone marrow without complication. The patient was given anti-inflammatory medication not usually given during transplantation which might have contributed to graft loss. Given this case, there is the possibility that graft loss may occur for other trial participants. Graft loss is seen under standard of care conditions as well in <1% of patients, and we currently do not have enough patient experience to know if there is an increased risk for trial participants as compared to standard of care transplantation.

6.3.3.1.2 Risks Related to the Transplant Procedure.

- Allogeneic transplant is associated with the following risks:
 - **Graft-versus-host disease (GVHD):** GVHD condition is when the donor cells you receive attack some of your tissues and organs. Your study doctor can describe the possible manifestations of GVHD to you.
 - **Bleeding:** Your platelet count will drop leaving you at risk for bleeding. In some cases, bleeding can be a fatal complication. You will receive transfusion of platelets to minimize the risk of bleeding.
 - **Veno-occlusive Disease (VOD):** High dose chemotherapy, radiation therapy and medications used to prevent graft versus host disease can cause veno-occlusive disease. Veno-occlusive disease can result in liver failure and in some cases, death.
 - **Mouth Sores and Diarrhea:** The large doses of chemotherapy and radiation cause irritation in the lining of the mouth and intestines, resulting in painful mouth sores and diarrhea.
 - **Capillary Leak Syndrome:** After chemotherapy and radiation, the blood vessels may become “leaky” and fluid enters your abdomen, lungs, and other tissues. Capillary leak syndrome can be difficult to manage if extra fluid enters your lungs and makes it hard to breathe. Capillary leak syndrome can be a fatal complication. .
 - **Organ Damage:** You may experience life-threatening or long-term injury to the heart, lung, kidneys, brain or liver damage as a result of your transplant.

- **Infection:** The chemotherapy and radiation treatments, GVHD prophylaxis and treatment, as well as allogeneic HSCT are associated with increased risk for infections. You will be given medications as well as instructions to minimize the risk of infections. You will be monitored very closely for infections and treated promptly if there is suspicion of or documented infection.
 - **Antimicrobial Prophylaxis:**
 - Side effects may include nausea, vomiting diarrhea, loss of appetite, or rash
- **Late Effects:** You may experience late effects that occur months to many years after your transplant.
 - You may have problems with your thyroid gland that require you to take thyroid medication.
 - You may get cataracts earlier in life compared to a person who has not had a transplant. If you develop cataracts (cloudiness in the eyes) they may need treatment.
 - Your kidneys could be affected and cause anemia (low red blood cell count) or high blood pressure.
 - You may develop a second cancer as a result of the chemotherapy, radiation and/or underlying disease. If secondary cancers happen, they generally do not develop until 10 to 15 years after your transplant.
 - Cancer therapies have the potential to accelerate the development of chronic health problems such as cardiovascular disease, cognitive changes, pulmonary, liver and kidney problems.
- **Unforeseen Risks:** New risks might appear at any time during the study that are different from the risks listed in this consent form. We will promptly tell you of any new information that may affect your decision to participate.
- **Additional Expected Risks:** Adenovirus infection, alopecia, anemia, constipation, cystitis, haemorrhagic, cytomegalovirus infection, diarrhea, Epstein-Barr virus infection, febrile neutropenia, fever, graft failure, hypertension, hypotension, incontinence, lymphoproliferative disorder, mucosal inflammation, nausea, neuropathy peripheral, edema, pain, rash, second primary malignancy, thrombocytopenia, thrombotic microangiopathy, veno-occlusive liver disease, and vomiting.

6.3.3.2 CD19-CAR T cell Therapy Associated Toxicities

CD19 CAR T gene-modified cells have been given to hundreds of individuals but CD22 CAR T cells have been given to far fewer individuals. There is still a lot we may not know and there may be risks that we cannot predict. At this time, over 35 adults and 5 children have received CD19/CD22-CAR T cells.

Dramatic and rapid expansion of CD19-CAR T cells is often associated with toxicity, including cytokine release syndrome and neurotoxicity.

6.3.3.3 Cytokine release syndrome (CRS)

Adult patients with ALL have been shown to develop severe CRS¹⁷, only in patients with morphologic evidence of disease (>5% blasts in BM) at the time of T cell infusion. CRS will be graded using the American Society for Transplantation and Cellular Therapy (ASTCT) consensus grading²³.

Severe CRS is defined by the presence of one of the following clinical and laboratory parameters:

- Hypotension: SBP<90 refractory to IV fluids requiring at least one vasopressor
- Respiratory distress/hypoxia requiring increasing supplemental oxygen or ventilatory support
- Acute coronary syndrome (ACS) with positive troponin, clinically significant arrhythmia, and/or ECG changes

6.3.3.4 Immune effector cell (IEC)-associated neurotoxicity syndrome (ICANS)

Patients with severe CRS may develop neurological complications including confusion, delirium, expressive aphasia, obtundation, myoclonus and seizure-like activity¹⁷. Some of the previously treated adult ALL patients required ICU stay due to these side effects, and few patients required intubation. All these side effects were observed in patients with morphologic ALL at the time of T cell infusion. Patients will receive levetiracetam (Keppra) for seizure prophylaxis at least 2 days prior to T cell infusion and be monitored closely for these side effects. In case to allergic reactions to levetiracetam (Keppra), alternative anti-seizure medications will be used under the guidance of the Neurology team. Neurology service will be consulted immediately, and the following management algorithm will be utilized upon the first suspicion of any neurological side effects. Baseline brain imaging should be conducted whenever feasible. Neurotoxicity, which is also referred to as immune effector cell (IEC)-associated neurotoxicity syndrome (ICANS) will be graded using the ASTCT consensus grading and the associated Immune Effector Cell-Associated Encephalopathy (ICE) score²³.

6.3.3.5 Lentiviral Gene Therapy Risks

6.3.3.5.1 Effect of DNA Integration

Most DNA integration is expected to cause no harm to the cell or to the patient. However, there is a chance that DNA integration might result in abnormal activity of other genes. In most cases, this effect will have no health consequences. However, in some cases, abnormal activity of a gene may cause unpredictable harm such as the development of cancer.

Discussion of delayed adverse event, leukemia-like malignancy, occurring in human studies:

It is important that you know about some cancers that occurred in another gene therapy research study. Clinical studies were conducted in France and United Kingdom to treat a disease called X-linked Severe Combined Immunodeficiency (SCID). Years after receiving cells that were modified by a retroviral vector, a significant number of the children in this small study developed a leukemia-like malignant disease (cancer). One child died from the cancer. A group of experts in this field studied the results from tests performed on these children's blood cells. They concluded that cancer was caused by the retroviral vector DNA. Still, most of the children with X-linked SCID who have received experimental gene therapy have not been found to have cancer at this time. Although they appear healthy, we still do not know whether they, too, will develop cancer.

Risk of malignancy for this study:

We do not know if the retroviral vector used in this protocol might cause cancer. However, you should be aware that the DNA contained in retroviral vectors will integrate into your DNA and that under some circumstances; this has been known to cause cancer months to years later.

6.3.3.6 Lentiviral Infection Risks

The cells we will be giving you have a type of virus (lentivirus) put into them along with the CD19/CD22 car gene. Although this lentivirus is not active, there is the rare possibility that it may regain the ability to make copies (replication competent lentivirus). Replication competent lentivirus has never been observed in human gene transfer studies to date. Lentivirus gene insertion could genetically alter your lymphocytes causing you to develop another type of cancer in your blood cells. This has been observed in studies inserting genes into stem cells, but has not yet been observed in clinical studies inserting genes into mature T-cells, like this study. You will be carefully monitored for 5 years after cell infusion for replication competent lentivirus and for 15 years after cell infusion for new cancers caused by the lentivirus.

The virus used in this study was built in a lab from parts of the **HIV** virus. While there is no chance you could contract **HIV** from the study treatment, future **HIV** tests may yield a “false positive” because the test detects virus segments that are similar on the two viruses.

6.3.3.7 Risk of Graft Failure

There is a risk of graft failure with allogeneic hematopoietic cell transplant procedures as well as an unknown risk of graft failure with administration of this novel CAR T therapy in the post-transplant setting.

6.3.4 Graft-Versus-Host Disease (GVHD)

Acute GVHD can affect the skin, the gastrointestinal tract or the liver. Symptoms may include

- A rash, with burning and redness of the skin. This may erupt on the patient’s palms or the soles of the feet, and often involves the trunk and other extremities as well. The skin may blister, and in severe cases of GVHD, the exposed surface of the skin may flake off.
- Nausea, vomiting, abdominal cramps, loss of appetite and diarrhea indicate involvement of the gastrointestinal tract.
- Jaundice (yellowing of the skin or eyes) may indicate that GVHD has injured the liver. Abnormalities of liver function would be noticed on blood test results.

6.3.4.1 Assessment, Staging and Grading of Acute GVHD

An assessment of aGVHD should be performed at all visits indicated by the Schedule of Events: Recipient in **Section 6.1**.

Please see **Appendix C** for information on the assessment, staging and grading of acute GVHD.

6.3.5 Non-Conforming Products

If a product(s) does not meet the requirements for subject infusion, for whatever reason (manufacturing failure, out-of-specification, lost or damaged product in transit, bag failure, etc), the subject will be monitored, and the investigators will determine the most appropriate treatment plan.

Specifically, if a product is out-of-specification (OOS), the Sponsor-Investigator will decide if the subject will be.

7 ADVERSE EVENTS AND REPORTING PROCEDURES

7.1 Adverse Event Definitions

7.1.1 Adverse Event

An adverse event can be any unfavorable and unintended sign or symptom, including abnormal laboratory findings, or disease, that is temporally associated with the use of a drug, and does not imply any judgment about causality. For this study, AEs will include events reported by the subject, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign, or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. An adverse reaction is any event that is caused by a drug or device, ie, possibly-, probably-, or definitely-related to the use of the drug or device. All adverse events will be graded according to NCI CTCAE v5.0 and **Appendix B**.

Except as otherwise explicitly defined within this section, **this also includes all events of clinical deterioration such as tumor relapse, recurrence, or upstaging, or new cancers.**

An abnormal laboratory value will be considered a reportable, recordable AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study therapy
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention, except for Grade 1 electrolyte changes or prophylactic therapies
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- Is a hematologic abnormality, including WBCs, hemoglobin, neutrophils, and platelets that constitutes a change in grade or attribution from baseline (e.g., worsens from baseline Grade 1 to Grade 2) during the first 28 days, or Grade 3 or 4 greater than 30 days post CD19/CD22-CAR T infusion.

7.1.2 Serious Adverse Event

Serious adverse events (SAEs) are defined per [the FDA definition at 21CFR§312.32\(a\)](#) and [ICH GCP E6](#). An adverse event is considered "serious" if, in the opinion of the PD, investigator, or sponsor, it results in ANY of the following:

- Death
- Life-threatening adverse event with an **actual and immediate risk of death** [21CFR§312.32(a)]
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Event jeopardizes the subject and may require medical or surgical intervention to prevent one of the outcomes listed here
- Hospitalization as an inpatient hospital stay equal to or greater than 24 hours
 - Hospitalization is used as an indicator of the seriousness of the AE and should **ONLY** be used for situations where the SAE truly fits this definition and **NOT** for

hospitalizations associated with less serious events (e.g., hospital visits where a patient is admitted for observation or minor treatment [e.g., hydration] and released in less than 24 hours). Furthermore, hospitalization for pharmacokinetic sampling is not an SAE, and therefore is not to be reported either as a routine AE or SAE. ([NCI Guidelines: Adverse Event Reporting Requirements](#))

Deaths occurring after the 30-day window due to disease progression or causes unrelated to study interventions will not be recorded as an SAE, but will be documented as a study outcome

7.1.3 Suspected Adverse Reaction

A suspected adverse reaction is any adverse event for which there is a reasonable possibility that the investigational therapy caused the adverse event (see [Section 7.3.3](#) for attribution). For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the investigational therapy and the adverse 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.

7.1.4 Unexpected Adverse Reaction

An adverse event is considered "unexpected" if it is not listed in the protocol or informed consent documents as a possible risk; or is not listed at the specificity or severity that has been observed. "Unexpected" also refers to adverse events that are mentioned in the protocol or informed consent documents as occurring with a class of investigational agents or as anticipated from the pharmacological properties of the investigational agent but are not specifically mentioned as occurring with the particular study agent under investigation.

7.2 Adverse Events of Special Interest

7.2.1 Second versus Secondary Malignancy

7.2.1.1 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). A second malignancy is by definition a serious adverse event (SAE), and will be recorded and reported accordingly.

7.2.1.2 Secondary Malignancy

In the context of a clinical study, a secondary malignancy is a cancer caused by chemotherapy, radiation, or the investigational agent/intervention. A secondary malignancy is not considered a metastasis of the initial neoplasm, but nonetheless, a secondary malignancy is by definition a serious adverse event (SAE), and will be recorded and reported accordingly. Secondary malignancies are usually described as one of the following:

- Leukemia secondary to oncology chemotherapy [eg, acute myelocytic leukemia (AML)]
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

7.2.2 Progressive Disease

Progressive disease will be reported by the clinical signs or symptoms of disease progression. If the event is Grade 5 fatal and signs/symptoms of disease progression are not available / informative for the event, the CTCAE v5.0 preferred term “Disease Progress” may be used.

7.2.3 Pregnancy

7.2.3.1 Maternal Exposure

If a female subject becomes pregnant during the course of the study, the study treatment should be discontinued immediately and the pregnancy reported to the Sponsor and the Stanford regulatory authorities (IRB, APB, DSMC).

Pregnancy itself is not regarded as an AE unless there is a suspicion that the study treatment under study may have interfered with the effectiveness of a contraceptive medication. However, as subjects who become pregnant on study risk intrauterine exposure of the fetus to investigational agents of unknown teratogenicity, any pregnancy occurring while the subject still has detectable CD19/CD22-CAR T cells in peripheral blood should be recorded and handled as an SAE using the CTCAE v5.0 preferred term Grade 3 “Pregnancy, puerperium and perinatal conditions - Other (pregnancy)”.

Congenital abnormalities or birth defects and spontaneous miscarriages should be recorded and handled as SAEs. Elective abortions without complications should not be handled as AEs. The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

7.2.3.2 Paternal Exposure

Male subjects should refrain from fathering a child or donating sperm for 4 months after the transplant preparative regimen and while CD19/CD22-CAR T cells are detectable in peripheral blood.

Pregnancy of the subject’s partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should, if possible, be followed up and documented. Pregnant partners of study participants must consent to data collection on an IRB-approved consent form before data collection can occur.

7.2.4 Adverse Events of Special Interest related to GVHD or Graft Performance

The following additional adverse events of special interest (AESI) are included in this study:

- Any grade acute GVHD
- Any grade chronic GVHD
- Hemophagocytic lymphohistiocytosis (HLH)
- Post-transplant lymphoproliferative disorder (PTLD)
- Grade 2 or higher infections (within the first year following Orca-T and CAR-T)
 - With the exception of thrush, UTI, uncompleted respiratory viruses, minor skin infections
- Graft failure
- Grade 3 or higher bleeding events

For all AESI, the overall specific diagnosis, not the individual laboratory abnormalities or clinical signs/symptoms should be recorded in the AE log.

7.2.5 Adverse Events of Special Interest Related to Gene Therapy

Subjects who receive CD19/CD22-CAR T cells will have Long Term Gene Therapy evaluations at least annually for 15 years post-infusion. All AESI related to Gene Therapy safety monitoring should be recorded for the 15-year period, including:

- Any new malignancies
- New incidence or exacerbation of a pre-existing neurologic disorder
- New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder
- New incidence of a hematologic disorder
- New incidence of infections that occur beyond one year will be collected only if they are potentially related to investigational CD19/CD22-CAR T cells

7.3 Classification of Adverse Events

7.3.1 Severity of Event

Adverse events will be graded in severity of event based on NCI CTCAE version 5.0 and **Appendix B** for CRS and ICANS and **Appendix C** for GVHD.

7.3.2 Attribution of Event

For this study, all recorded adverse events will assessed by the PI or delegated qualified designate for whether the adverse event was caused by / due to the study intervention(s). For every recorded AE, serious or otherwise, the Principal Investigator or qualified delegated research staff will consider an attribution to each investigational agent separately in accordance with the definitions below.

If additional information becomes available, it is permissible to update an AE's attribution (e.g., confirmatory evidence of disease relapse or remission).

• Definitely Related. There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal of the study intervention (dechallenge) should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary. • Probably Related. There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal (dechallenge). Rechallenge information is not required to fulfill this definition. • Possibly Related. There is some evidence to suggest a causal relationship (eg, the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (eg, the participant's clinical condition, other concomitant events). Although an adverse event may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related," as appropriate.	These are treated as "Related" as per Section 7.1.3
• Unlikely to be related. A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (eg, the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (eg, the participant's clinical condition, other concomitant treatments). • Not Related. The adverse event is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.	These are treated as "Not Related"

Note that adverse events can be determined related to both the investigational agent(s) and/or the study procedures.

In addition, for adverse events determined "Not Related" to the study intervention(s), the Principal Investigator or qualified designate will attribute the event to the study or procedures according to the definitions above. Note that adverse events can be determined related to both the intervention(s) and/or the study / procedures.

7.3.3 Expectedness of Event

The Principal Investigator or qualified designate will also assess all recorded adverse events on basis of event severity, frequency (if applicable/assessable), and the established product risk information described within the product Investigator Brochure; the FDA-approved product labeling (if an approved agent); and/or this protocol document, as to whether the events are "expected" or "not expected" relative to the study interventions and/or the study / procedures.

Note that unexpected adverse events may have reporting requirements as described elsewhere in this section.

7.3.4 Time Period of Event Assessment and Follow-up

All SAEs and AESIs will be followed until resolved or stable. Non-serious AEs will be followed until resolution to baseline or until at least 42 days after investigational agent administration, whichever is shorter.

7.4 Potential Adverse Events and Risks

Potential adverse events and risks described in Section 1.3.1 Known Potential Risks and in the informed consent form are considered expected at the severity and frequency discussed.

In addition, risks described in the package insert of FDA-approved drugs used in this study are considered expected. Safety updates provided by the IND Sponsors of cross-referenced INDs, either via public disclosure in scientific publications or presentations or via private communication, will be incorporated into this protocol but may be considered when determining expectedness ahead of formal protocol amendment. Procedural risks described in Section 1.3.1 or in the consent form are considered anticipated.

7.5 Adverse Event Monitoring and Collection

7.5.1 Donor Adverse Event Collection

Adverse events, as defined in **Section 7.1**, will be collected in donors from the start of unmobilized apheresis through 3 days after the mobilized apheresis collection.

Only adverse events attributed as possibly, probably, or definitely related to study procedures will be collected for donors. Only unexpected adverse events will be documented in the EDC.

Due the high number of unrelated Grade 1 events in this therapeutic setting, only Grade 2 or higher adverse events will be collected and recorded as AEs. However, adverse events that are Grade 1 and considered related to study treatment will also be recorded.

7.5.2 Recipient Adverse Event Collection

Adverse events, as defined in **Section 7.1** and **Section 7.2**, will be collected in recipients under the following time frames and guidelines:

- Between signing informed consent and start of transplant preparative regimen: Only adverse events attributed as possibly, probably, or definitely related to study procedures will be collected in recipients.
- Toxicities attributed only to the transplant preparative regimen and occurring prior to Day 0 will not be collected.
 - On Day 0 prior to receipt of Orca-T, all medical conditions present or ongoing should be recorded as Baseline/"Cycle 0" conditions.
- From Day 0 to Day 42 post-CD19/CD22-CAR T cell infusion: All AEs that are new, a change in grade from baseline, or a change in attribution from baseline will be collected in

recipients. If a recipient was unable to receive donor CD19/CD22-CAR T cells, the collection window for all adverse events will be Day 42 post-T_{con} infusion.

- From Day 42 post- donor CD19/CD22-CAR T cell infusion until Day 100 post-Orca-T: All adverse events of special interest (AESI, see [Section 7.2](#)), and cytopenias (e.g., neutropenia, thrombocytopenia, and anemia) will be collected in recipients.
- Beyond Day 100 post-Orca-T: only serious adverse events attributed as possibly, probably, or definitely related to the investigational cell products and AESI (see [Section 7.2](#)) will be collected in recipients.
- All non-hematologic laboratory AEs will not be collected with the exception of those deemed clinically significant.

7.5.3 Case Report Forms for Adverse Event Reporting

Adverse events, both serious and non-serious, will be recorded within the medical record in a log format providing the unique subject identifier, event name, date of occurrence, date of change in grade, severity, attribution to donor CD19/CD22-CAR and attribution to Orca-T, outcome, and any action taken. The PI or designee review and sign off on AE logs should occur within the time frames outlined in CCT SOP 'Adverse Event Review and Documentation'.

SAEs will be reported to the IND Sponsor and DSMC through CCTO-Safety using the electronic CCTO SAE eCRF (2 agents).

Adverse event data will also be entered into REDCap to facilitate statistical analysis.

7.6 Adverse Event Reporting

Both Serious and Non-Serious Adverse Events will be clearly noted in source documentation and listed on study specific AE logs. The Principal Investigator or designee will assess each AE to determine whether it is unexpected according to the Informed Consent, Protocol Document, or related to the investigation. **Reportable adverse events** are defined as AEs that occur during the collection windows described in [Section 7.5](#) based on relatedness (attribution) to the study agents; expectedness, severity (Grade 1 to 5), seriousness (Yes/No), or any other aspect of the investigation.

- Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (eg, cough, runny nose, sneezing, sore throat; and head congestion should be reported as "upper respiratory infection").
- For SAEs, further work-up or observation that results in a change in relatedness, expectedness, or severity should be promptly communicated in a follow-up report.

7.6.1 Second vs Secondary Malignancy

7.6.1.1 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is NOT a metastasis from the initial malignancy). A second malignancy is by definition a serious adverse event (SAE), and will be recorded and reported accordingly.

7.6.1.2 Secondary Malignancy

In the context of a clinical study, a secondary malignancy is a cancer caused by chemotherapy, radiation, or the investigational agent/intervention. A secondary malignancy is not considered a metastasis of the initial neoplasm, but nonetheless, a secondary malignancy is by definition a serious adverse event (SAE), and will be recorded and reported accordingly. Secondary malignancies are usually described as one of the following:

- Leukemia secondary to oncology chemotherapy [eg, acute myelocytic leukemia (AML)]
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

By definition, secondary malignancies are adverse events that are both serious and related to the research, and should be anticipated to be an Unanticipated Problem (UP) and reported accordingly. If also determined to be related to the study treatment, and not described by the Investigator Brochure or package insert as known to be associated with the study agent, the secondary malignancies may also necessitate Expedited Reporting to FDA, eg, an IND Safety Report to the IND.

7.6.2 Progressive Disease

Progressive disease will be reported by the clinical signs or symptoms of disease progression. If the event is Grade 5 fatal and signs/symptoms of disease progression are not available / informative for the event, the CTCAE v5.0 preferred term “Disease Progress” may be used.

7.6.3 Unanticipated Problems (UPs)

- All **Unanticipated Problem (UPs)** associated with the use of a drug, biologic, or device will be reported as follows.
 - Note that for the purposes of Stanford IRB Unanticipated Problem reporting, subjects are considered to be study participants when consented, i.e., events during screening and/or pre-treatment through that subject’s official end of study participation may qualify as reportable.
 - To the Stanford IRB, by the Stanford Protocol Director, if an adverse event meets the Stanford IRB’s current definition of an Unanticipated Problem (UP) as specified by the IRB document “Events and Information that Require Prompt Reporting to the IRB” (GUI-P13), per the timeframes defined therein. See also Section 9.3 Data and Safety Monitoring Plan. In addition to event triggered expedited UP reports, all UPs should be summarized in the Continuing Review, with, as applicable, a discussion of any change in assessment of risk (ie, different than previously described).
 - To the SCI Data and Safety Monitoring Committee (DSMC), by submission to CCTO-Safety / OnCore as an SAE (see SAE reporting to DSMC above. See also the SCI DSMP).

Subsequent follow-up reports, whether to SAEs or UPs, will be reported as required by the receiving entities.

7.6.4 IND Sponsor Reporting Criteria

Reportable adverse events, based on relatedness (attribution) to the study agent; expectedness, severity (Grade 1 to 5), seriousness (Yes/No), or any other aspect of the investigation, will be reported as described below.

- Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (eg, cough, runny nose, sneezing, sore throat; and head congestion should be reported as "upper respiratory infection").

The Principal Investigator must **immediately** (within 24 hours of knowledge of event) report to the Sponsor-Investigator (IND Holder), using the CCTO SAE eCRF, any serious adverse event, whether or not considered drug related or expected. The PI must include an assessment of relatedness and expectedness to each investigational agent administered, along with supporting documentation (e.g. lab reports, progress notes). The Principal Investigator is responsible for promptly providing the Sponsor with any information needed to determine whether the SAE requires submission of an IND Safety Report.

Subject death due to disease progression or unrelated to study procedures or investigational agents does not require immediate reporting via CCTO SAE eCRF but should be communicated to the IND Sponsor-Investigator using regular team communications as described in [Section 9.3.1](#).

Pregnancy of a female participant within 4 months after transplant preparative regimen or while CD19/CD22-CAR T cells are still detectable in her peripheral blood must be immediately (within 24 hours of knowledge of event) reported to the IND Sponsor-Investigator.

Non-serious adverse events must be recorded in a timely manner and reported to the IND Sponsor-Investigator to facilitate preparation of IND Annual Reports.

Contact information for IND Sponsor-Investigator:

Melody Smith, MD, MS
300 Pasteur Drive, H0101
Stanford, CA 94305
T: 650 723 0822
F: 650 736 8092
melodysm@stanford.edu

7.6.5 FDA Reporting Criteria

7.6.5.1 IND Safety Reports

The IND Sponsor-Investigator will be responsible for reporting to the FDA any serious unexpected suspected adverse reaction related to the investigational regimen (SUSAR or IND Safety Report), as per [21CFR§312.32](#), within 7 days (for a life-threatening or fatal event) or 15 days (for other SAEs) of the IND-holder's determination that the SAE meets the criteria for an IND safety report. IND Safety Reports will use the Stanford Cancer Cell Therapy (CCT) Safety Report template or FDA MedWatch Form 3500a. The IND Sponsor-Investigator is responsible for responding to any FDA information request but may invite the PI to participate in discussion with FDA reviewers as needed.

The PI is responsible for providing supporting documentation and regular updates to the IND Sponsor-Investigator until the event is resolved.

7.6.5.2 IND Annual Reports

A summary of all collected adverse events, serious and non-serious, will be provided to the FDA at least annually in the IND Annual Report. The IND Sponsor-Investigator is responsible for submitting the Annual Report as specified by [21CFR§312.33](#).

7.6.5.3 Non-Conforming Cell Therapy Product Reports

A sample from all products that are non-conforming or do not meet release specifications will be used to conduct an out of specification investigation, and the remainder either disposed of according to the GMP facility biohazardous material disposal SOP or the FDA will be contacted to petition whether the product is suitable for infusion. The ultimate decision whether to release a product for infusion is the responsibility of the IND Sponsor-Investigator, but the PI has ultimate responsibility for deciding whether administering a non-conforming product is in the best interest of the study subject and for properly informing the subject of the risks.

The summary of all products manufactured, including those that did not meet release criteria or were otherwise not infused, will be included in the annual IND report to the FDA.

All HCT/P deviations involving 351 cell products will be reported using MedWatch Form FDA 3500A according to FDA publication “Guidance for Industry: MedWatch Form FDA 3500A: Mandatory Reporting of Adverse Reactions Related to Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps) available at:

<http://www.fda.gov/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Tissue/ucm074000.htm>.

7.6.5.4 Action Plan for Positive Sterility Result on Previously Administered Allograft Product

In the unlikely event that a positive sterility test result is obtained after distribution of a cell product and administration of the product to the subject, the following steps will be initiated IMMEDIATELY:

- a. Clinical Laboratory personnel will notify the manufacturing facility and the Director of Manufacturing and Process Development at Stanford Center for Cancer Cell Therapy of the test results.
- b. The manufacturing facility personnel or the Director of Manufacturing and Process Development will notify the Principal Investigator at 650-721-2785. The IND Sponsor-Investigator will also be immediately notified. Both will be updated with any substantive changes, including the final report on the identification and sensitivity from the positive sterility test. The manufacturing facility QS Director or designee will determine the need for quality improvement based on the nature and extent of the incident.
- c. If the manufacturing facility or Director of Manufacturing and Process Development is unable to reach the Principal Investigator within 15 minutes, he will contact inpatient attending physician caring for the subject on the hospital service via hospital page with direct communication. NOTE: The Sponsor; Principal Investigator; and/or designee will contact the attending physician, who will determine the extent of the work-up of a positive

culture in consultation with appropriate infectious disease consultants, as well as determine an appropriate action treatment plan.

- d. The Principal Investigator/attending physician will discuss the positive results with the subject, and specify the clinical therapy, antibiotic regimen and/or monitoring plan.
- e. A contaminated sample of a product that has been administered to a subject will be handled in the same fashion as a Grade 4/5 toxicity. The Principal Investigator will be responsible for notifying the IRB and APB via an Unanticipated Problem (UP) report within 5 working days, and the Sponsor will notify the FDA via an expedited 7-day IND Safety Report.

In addition to the above, appropriate Safety reporting will be done as per the manufacturing facility's SOP for Sterility Testing. A sample of each product is retained by Quality Systems and will be sent to the Microbiology Laboratory for repeat testing and speciation. An Out-of-Specification (OOS) Investigation will be conducted by the Quality Systems staff of the manufacturing laboratory including root cause analysis, review of viable environmental monitoring results collected at the time of manufacturing on personnel, equipment, and reagents. Whether or not attribution is established, a formal Corrective and Preventive Action plan will be issued by the manufacturing facility Quality Systems staff and appropriate remediation will be performed including retraining of manufacturing personnel, elimination of any contaminated reagents and re-cleaning of the production facility followed by viable microbiological monitoring to establish effectiveness of cleaning.

7.6.5.5 Action Plan for Positive Sterility Result on Previously Administered Donor Derived CD19/CD22 Product

If a CD19/CD22 CAR T product eventually tests positive in the 14D sterility or 42D fungal assay post-product infusion, the LCGM manufacturing facility will immediately notify the individuals listed below: CCT Manufacturing Director, CMC Operations Manager, CCT Regulatory team, and Study PI. A retain final product vial, or second clinical dose if available, may be re-submitted for sterility testing as part of a root cause analysis investigation.

7.6.6 Stanford Reporting Criteria

All SAEs, regardless of attribution, collected during the windows described in Section 7.5 and all subsequent follow-up reports will be reported to the Stanford Cancer Institute Data and Safety Monitoring Committee (DSMC) using the CCTO SAE eCRF. The SAE eCRF must be signed by the PI or delegated investigator and will be sent by **secure** email to CCTO-safety@stanford.edu. CCTO-Safety will process the SAE report into OnCore, and the DSMC will review the event.

7.6.6.1 APB Reporting

Unexpected SAEs Grade 3 and above that are possibly, probably, or definitely related to donor CD19/CD22-CAR T cells or Orca-T infusion will be promptly reported to Stanford Administrative Panel on Biosafety (APB) via eProtocol Report. All FDA communications regarding IND Safety Reports or positive sterility events will also be promptly reported to APB via eProtocol Report.

All other AEs and SAEs will be reported to Stanford APB at least annually by submitting a copy of the IND Annual Report once it is available.

7.6.6.2 IRB Reporting

Events meeting the IRB definition of 'Unanticipated Problem' (UP) as defined in IRB document GUI-P13 will be reported to the IRB using eProtocol within 10 working days of PI knowledge of event, or within 5 working days for deaths or life-threatening experiences.

A summary of all adverse events will be reported to Stanford IRB at least annually by submitting a copy of the IND Annual Report once it is available.

7.6.7 Orca Bio Reporting

7.6.7.1 Adverse Event Reporting

To ensure that Orca Bio (Orca) is aware of relevant safety data, all SAEs (as defined in 21 CFR 312.32) shall be reported to Orca by emailing a completed Form FDA 3500 within forty-eight (48) hours of first knowledge of the event by Stanford. All SAEs will be followed until resolution or stabilization of the event, the subject dies, is lost to follow-up, or starts a new therapy. As new information becomes available that is clinically relevant to the case, Stanford will promptly forward this follow-up information to Orca.

Contact information for Orca: OrcaSafety_IIT@orcabio.com

In addition, once each calendar quarter, Stanford shall also provide a report containing a list of all adverse events observed during the Study to Orca. These quarterly reports of all adverse events shall be sent via the e-mail provided above.

7.6.7.2 Safety Reporting

Orca will provide Stanford with any data and safety monitoring reports related to the Product during the Study and for at least two (2) years following completion of the Study that may: (i) affect the safety and welfare of current or former Study participants or (ii) influence the conduct of the Study. Stanford will submit such reports to the IRB as required.

7.7 Adverse Event Records

The PI or designee will retain adverse event source data, supporting documentation of attribution and seriousness, and copies of official adverse event reports or SAE CRFs, as well as documentation of informal communications (such as telephone calls or emails) in accordance with the current version of Stanford School of Medicine standard operating procedure SOP-005 "Identifying and Reporting Adverse Events".

8 CORRELATIVE / SPECIAL STUDIES

8.1 Correlative Studies Background

The proposed correlative studies aim to advance our understanding of donor CD19/CD22 CAR T cells administered following Orca-T by investigating the pharmacokinetics and pharmacodynamics of these cells. We will also evaluate analyses that foster discovery-based insights of donor CAR T cell therapy.

8.2 Laboratory Correlative Studies

8.2.1 Sample Collection Schedule

The samples to be collected and schedule for sample collection is detailed in **Appendix E**. Every effort will be made to collect correlative samples as described in **Appendix E**, but changes in tests, schedules, sample volume limitations and/or conduct of apheresis, and conflicts with schedules or patient conditions may result in missed or altered sample collection. These variations will be noted but will **NOT** constitute a protocol deviation given the exploratory nature of correlative testing. At the discretion of the principal investigator, correlative samples may be omitted if not applicable to the subject's disease or not in the best interest of the subject. Omitted correlative samples for these reasons should not be considered a protocol deviation.

8.2.1.1 Post-Treatment Sample Collection

Samples will be collected beyond one-year post-treatment at 18 months, 24 months, and then annually. Samples will only be collected if subjects present for in-person visits.

Additionally, we will stop further sample collection if the CAR becomes undetectable.

8.2.2 Blood Drawing Limits for Research Purposes

The maximum amount of blood that may be drawn per day from adult subjects is 2.5% of total blood volume (192 to 200 mL). The volume to be drawn per day for research purposes will comply with institutional policy. Subjects may undergo a small volume apheresis (approximately one to two blood volumes) in lieu of standard blood draw to obtain peripheral blood lymphocytes for correlative studies, including Day 28 ($\pm$ 1 week).

8.2.3 Specific Correlative Studies

CAR T cell therapy targeting a single tumor associated antigen has mediated remissions in B cell leukemia; however, the complex interplay of tumor, T cell and intrinsic CAR properties that influence these outcomes are not well understood. The clinical experience to date has demonstrated variable patterns of tumor remission and CAR T cell efficacy and persistence. We aim to utilize this study as an opportunity to collect correlative data that will permit extensive study of both the B cell and T cell compartment prior to and following CAR T cell therapy. We aim to integrate multi-dimensional technologies to permit complex analyses of the apheresis product, the CAR T cell product pre-infusion and *in vivo* expanded CAR T cells following antigen encounter. We additionally aim to investigate properties of B cell reconstitution and phenotypically study the effect of combination ORCA T cells and CAR T cells in these patients.

Specific aims for the correlative analyses of the study are noted below:

1. Measure persistence of T_{cons}, T_{regs}, and donor CD19/CD22-CAR T cells in the blood, bone marrow and CSF
2. Investigate donor CD19/CD22CAR T cell properties correlated with CAR T cell efficacy and persistence
3. Evaluate the impact of donor CD19/CD22-CAR T cells on immune reconstitution after Orca-T infusion including its impact on numbers of circulating B cells and bone marrow
4. Assess the impact of the donor CD19/CD22-CAR T cells on the genomic profile of the patients with CAPP-seq compared to historic controls

5. Investigate cytokine signature of the donor CD19/CD22-CAR T cells as compared to CD19/CD22-CAR T cells from autologous sources
Assess the impact of the donor CD19/CD22-CAR T cells on the ALL-stem cell and the bone marrow microenvironment
6. Evaluate whether donor CD19/CD22-CAR T cells traffic to GVHD target organs
7. Assess the impact of the intestinal microbiome, including bacterial taxa, metabolic pathways, and bacterial metabolites, on expansion and persistence of CAR T cells as well as on maintenance of B cell aplasia
8. Fecal samples may be collected to identify alterations in microbiota diversity, bacterial taxa, and genes correlated with complications of CAR T-cell therapy, including cytokine release syndrome and neurologic toxicity.

8.2.3.1 Persistence (Correlative Studies 1 & 2)

Due to the nature of these studies, it is possible that expansion of all T cells, genetically modified T cells and specific T-cell clones will be observed as tumor reactive T-cells proliferate in response to tumor antigens. Therefore, care will be taken to track T-cell persistence both immunologically and molecularly. We will use flow cytometry to track the CAR by using anti-FMC63 scfv anti-idiotypic antibody at the time points outlined in the correlative table in Appendix E. We will also assess the T cell compartment and study the CAR T cell proliferation, naïve and memory status. Molecularly we will measure the copy number for the genetic modification by qPCR and use scRNA Seq to cell fate map. We will include the BD Trucount™ Tubes in our flow cytometry assay to ensure that we can obtain absolute count measurements of each of the cell types. Cells from the peripheral blood, bone marrow and cerebrospinal fluid will be monitored as available and per the correlative table Appendix E.

8.2.3.2 Immune Reconstitution (Correlative Study 3)

We will monitor immune reconstitution using stored peripheral mononuclear cells and bone marrow cells according to flow cytometry and mass cytometry panels methods developed and validated in the Correlative Science laboratory. Using these panels, we will investigate the T cells, including naïve, memory, Treg and iNKT cells as well as NK and myeloid cells. Using the B cell panel, we will investigate B cell reconstitution and progenitors in the bone marrow. In addition, ALL specific functional markers, such as CD10, TdT, RAG1, pSYk, pCreb, TSLP-R, are included in our analyses. High dimensional methods including but not limited to scRNA seq, ATAC seq, TCR seq will further be implemented to investigate the functional status and origin of the cells.

8.2.3.3 Genomic Profiling (Correlative Study 4)

The impact of the epigenetic status of the CAR T cell in association with clinical parameters, such as response to therapy and CAR T cell expansion, will be further characterized using by performing ATAC-seq (Assay for Transposase Accessible Chromatin with High-throughput sequencing). The method is already optimized and is being performed investigating others CAR T cells through the Cancer Correlative Science Unit.

8.2.3.4 Cytokine Signature (Correlative Study 5)

EDTA plasma samples will be collected and evaluated for plasma Cytokines as well as Capseq assays.

8.2.3.5 Tumor Microenvironment (Correlative Study 6)

Bone marrow samples will be obtained from the patients per the timepoints in the Correlative Table in Appendix E. We will perform CAR FACS as well as scRNA Seq on these bone marrow samples and assess the tumor microenvironment, including ALL cells, B, T and myeloid cells. This data will be compared to available data on the tumor microenvironment of bone marrow samples from patients who received autologous CD19/CD22 CAR T cells.

8.2.3.6 GVHD (Correlative Study 7)

If patients treated on this clinical protocol develop GVHD, then we will seek to obtain a biopsy. The tissue biopsy will be evaluated with scRNA Seq through the CCSU or Formalin-Fixed Paraffin-Embedded (FFPE) through the Department of Pathology to evaluate the GVHD target organ. These assays will enable us to assess for trafficking of CAR T cells and non-CAR T cells to the sites. This data will enable us to assess what cells are contributing to alloreactivity in patients who develop GVHD.

8.2.3.7 Intestinal Microbiome (Correlative Study 8)

Fecal microbiome samples will be obtained from patients per the timepoints in the Correlative Table in Appendix E. We will perform DNA extraction, 16S shotgun sequencing, metagenomic shotgun sequencing, and metabolomics/protein analysis on the fecal microbiome samples. Sequenced reads will be quality filtered and taxonomically assigned. Human reads will be computationally removed in order to limit our analysis to only the microorganisms present in the sample. Longitudinal fecal will be compared between those achieving CR, PR, and no response using an appropriate statistical approach such as linear discriminant analysis effect size to identify bacterial taxa and genes enriched in each of the three groups. CAR T-cell persistence over time will also be correlated to changes in diversity and relative abundance of microbiome taxa in the longitudinal samples.

This data will be used to assess impact of the intestinal microbiome, including bacterial taxa, metabolic pathways, and bacterial metabolites, on expansion and persistence of CAR T cells as well as on maintenance of B cell aplasia. These studies will be analyzed in conjunction with plasma metabolomics.

8.3 Laboratory Correlative Studies

8.3.1.1 Collection of Specimen(s)

Biospecimens will be collected based on the above schedule (**Section 8**) from patients who have provided informed consent (described in **Section 3**) to participate in the study. Clinical research coordinators (CRCs) assigned to the study will schedule participating patient appointments and coordinate the distribution of research kits to the site of the patient visits in collaboration with the BMT-CT Biobank team. Patient appointments with corresponding research sample collections (date, time, location) will be tracked and distributed weekly via the BMT-CT Biobanking Smartsheet schedule, accessible to the CRCs, BMT-CT Biobank, Principal Investigators,

correlative study team, and nursing staff. Weekly meetings ensure that the patient is consented to and given a de-identified study ID, the schedule is up to date, and processing samples is coordinated between the respective staff members.

8.3.1.2 Handling of Specimens(s)

Research samples will be collected by the nursing staff and attending physician (in the case of bone marrow). Once collected, the nursing staff will notify the CRCs and/or the BMT-CT The BMT-CT Biobanking team. The BMT-CT biobanking team will collect processed research kits at the various inpatient and outpatient sites according to the BMT-CT Smartsheet schedule. Research specimens will be distributed by the BMT-CT Biobanking team to the correlative sciences team for real-time analyses or processed by the biobank under the appropriate protocols ([link to protocol](#)) and stored in liquid nitrogen (cells) or -80C freezers (plasma, cell-free, fecal samples) until requested for future research purposes per the protocols indicated below. Each parent sample and derivative aliquots are accounted for, and its storage location and distribution are tracked in the OpenSpecimen database. Sample storage locations have keycard-controlled building access and are temperature monitored 24 hours a day, seven days a week, 365 days a year, with emergency backup power.

8.3.1.3 Shipping of Specimen(s)

Requests for samples from the BMT-CT for research purposes are made via the Smartsheet form in compliance with the processes outlined in the CCT5019 protocol section 4.3 and 5. Sample requests for research use not currently described within the CCT5019 protocol must either have a separate IRB protocol approved or a negative Human Subjects Research (HSR) determination. Samples from the BMT-CT Biobank requested and approved for research use are monitored and checked out in OpenSpecimen. In addition, requests and samples provided (amount and type of samples, patients being derived from, analyses being performed, and compliance with CCT5019) are documented and tracked by the BMT-CT Biobank via Smartsheet. Once approved, will be couriered on dry ice locally at Stanford by the BMT-CT Biobanking team to an agreed-upon location on the Stanford campus. The oversight committee must approve samples to be shipped outside of Stanford University, and analyses performed on the samples must comply with the IRB protocol. Samples will be de-identified, and no transfer of PHI will occur without the consent of the IRB and oversight committee. Temperature-sensitive samples will be shipped on dry ice by individuals trained in the shipping of dry ice and human samples and labeled according to such designations (i.e., UN 1845 label). Samples will be shipped via FedEx at the expense of the receiver (unless agreed upon otherwise) and tracked appropriately.

8.3.1.4 Timing of Assays for Specimens

After approval at the Cancer Correlative Science Unit (CCSU) Project Review Committee the assays will be prepared for the trial. The specimens will be obtained and banked in accordance with the validated protocol at the CCSU as depicted **Appendix E** and as outlined by the BMT-CT Biobank Guidelines.

One green top tube (10 ml of whole blood) will be provided to the CCSU for real-time flow phenotype and CART characterization. The remainder of the samples will be banked and processed in batches according to the CCSU workflow as noted below.

PBMCs from a 10 ml lavender top (EDTA) tube, and the PBMCs will be viably frozen to be used for additional flow- and mass cytometry assays.

PBMC isolated from EDTA tubes will be banked for qPCR, scRNA-seq, ATAC-seq, bulk DNA and RNA-seq. Plasma isolated from the EDTA tubes will be banked in the -80°C freezer for batched plasma cytokine determination and plasma metabolomics.

Fecal samples will be aliquoted and frozen in cryovials in the -80°C freezer. These samples will be used for metagenomic shotgun sequencing, and metabolomics.

8.3.1.5 Coding Of Specimens For Privacy Protection

At the time of consent, each patient is assigned a unique, de-identified study number used by the BMT-CT Biobank and correlative science unit team to identify the patient while protecting personal health information (PHI) and patient privacy. PHI is removed from the labels on the original research kit and collection tubes and destroyed appropriately. After processing the sample, via appropriate approved protocols, each derivative aliquot is inputted, assigned a unique, de-identified barcode label and tracked in OpenSpecimen (LIMS Database).

9 REGULATORY CONSIDERATIONS AND DATA REPORTING

9.1 Review and Approval of Protocol by IRB and SCI SRC

This protocol, the proposed informed consent, and all forms of information related to the study that will be provided to the subjects (e.g., questionnaires, handouts, written instructions, diaries, advertisements used to recruit subjects, etc.) will be submitted to, reviewed, and approved by the SCI Scientific Review Committee (SRC) and the Stanford Administrative Panels on Human Subjects in Medical Research of the Research Compliance Office (i.e., the Stanford IRB) and the Stanford Administrative Panel on Biosafety (Stanford APB) prior to initiation of the research. Any changes made to the protocol will be submitted as a modification and will be approved by the IRB and APB prior to implementation. The SRC will review all protocol amendments that include changes to study design, risk, sample size or study endpoints.

This study will be conducted in accordance with the iteration of the protocol that is currently IRB-approved at the respective clinical sites. The Stanford Protocol Director will disseminate to all participating Principal Investigators any revisions to the protocol (ie, protocol amendments), or changes to risk information for use in informed consents, and updated Investigators Drug Brochures or product information.

9.2 Protocol Compliance and Deviations

9.2.1 Compliance with the Protocol

No deviation or changes from the procedures and process described by the IRB-approved protocol, except those necessary to eliminate an immediate hazard(s) to trial subjects, or when the change(s) involves only logistical or administrative aspects of the trial (e.g., change in study monitor(s), change of telephone number(s)), will be knowingly permitted without review and approval by the IRB of record.

9.2.2 Protocol Deviations

Any deviation from the IRB-approved protocol, including those that eliminate an immediate hazard or are administrative in nature, will be documented and explained in the study site file. Events that meet the reporting requirements defined in the Stanford University HRPP Policy Guidance “Events and Information that Require Prompt Reporting to the IRB” [GUI-P13] will be reported to the Stanford IRB and APB within the defined timeframes. A summary of all deviations will be provided in the IND annual report and IRB continuing review.

All deviations will be reported in OnCore for review by the SCI Data and Safety Monitoring Committee (DSMC) as per the current SCI DSMC SOP.

9.3 Data and Safety Monitoring Plan

9.3.1 Clinical Team

The clinical research team will meet on a regular basis during dose escalation and when subjects are being actively treated on the trial to discuss cell manufacturing, toxicities, eligibility questions, trial accrual, and treatment needs. Decisions about dose level enrollment and dose de-escalation if applicable will also be discussed as study milestones approach. Members from the CCT Process Development and Manufacturing, Stanford CTF, and cell processing facilities will join as needed.

The Principal Investigator (PI) is responsible for monitoring the conduct of the study including oversight of safety and protocol compliance. The PI may delegate study tasks to qualified staff members as documented on the delegation of authority log, but the PI retains ultimate responsibility for the study conduct and data management.

All data will be collected in a timely manner and reviewed by the PI or a designated investigator. Adverse events will be collected and reported as outlined in [Section 7.6](#). Any safety concerns or new information that might affect either the ethical and or scientific conduct of the trial will be immediately reported to the IND Sponsor-Investigator, IRB, APB and DSMC.

The PI will review adverse event and response data for each subject to ensure safety and data accuracy and to identify any changes to the research necessary to ensure the appropriate measures and monitoring necessary for participant safety are present. PI review of adverse event logs will conform to the CCT SOP ‘Adverse Event Review and Documentation’.

Routine study monitoring of essential study documents by the Principal Investigator or delegated staff member will be conducted by BMT-CT at an estimated frequency of not less than once a year.

9.3.2 Data Safety Monitoring Committee

The Stanford Cancer Institute Data and Safety Monitoring Committee (SCI DSMC) will monitor study-related activities to determine whether the study has been conducted in accordance with the protocol, local standard operating procedures, FDA regulations, GCP, and SOPs. In addition, the DSMC will regularly review serious adverse events and protocol deviations associated with the research to ensure the protection of human subjects. Results of DSMC monitoring activities will be communicated to the PI, who has the responsibility to provide such reports to the

IRB of record and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as needed.

9.4 Data Management

Source documents for all research data will be retained in accordance with all applicable regulations and institutional requirements for data retention. These materials will be made available for monitoring and/or auditing by SCI DSMC, other monitoring body and/or regulatory agencies.

9.4.1 Data Management Plan

Source data for adverse events, study procedures, clinical laboratory tests, and disease response assessments will be recorded in the electronic medical record (EPIC). Additional study source documents, such as manufacturing batch records, chain of custody forms, regulatory filings, etc. will be stored in secure physical or electronic systems with appropriate safeguards. Paper study files will be kept in a locked, secure location. Electronic study data will be stored on encrypted, backed-up, password protected computers or cloud systems. Any study documents containing PHI will be stored in electronic systems designated safe for PHI, such as Stanford Medicine Box and Smartsheet.

The study data will be transcribed and retained in a REDCap electronic data capture (EDC) online database, with password-protected access and role-based controlled access to the study team and department data managers.

9.4.2 Departmental Data Repository

The Stanford division of Blood & Marrow Transplantation (“BMT”) collects as part of standard-of-care over 1000 data fields from each patient undergoing a hematopoietic cell transplant into a coded database. This is an electronic database known as the “BMT Core,” a 21 CFR§11-compliant system. The system is supported by the BMT Data Management Group. The collected data fields include, but are not limited to, detailed patient demographics, treatment assessments pre- and post-intervention, adverse events, study endpoints such as overall survival, event-free survival, time-to-disease progression, relapse, acute and chronic graft vs host disease (GvHD), chimerism, etc. This data is reported at designated time points to the federally-supported Center for International Blood and Marrow Transplantation Registry (CIBMTR), the governing body for this therapeutic modality, in accordance with licensure from the Foundation for the Accreditation of Cellular Therapy (FACT).

The BMT Data Management Group maintains all aspects of the BMT Core database for the collected information. Meetings are held weekly to ensure quality (QA) and compliance (QC). As part of quality processes, the BMT Division is formally audited at standardized intervals CIBMTR and FACT.

9.5 Site Documentation and Management

The following information will be maintained by the Principal Investigator or designated study staff, either in a study-specific location or centrally:

- Delegation of Authority Log, indicating study role, on-study date (date of first research-related procedure or scan), and off-study date
- Training Logs to document appropriate training of research staff for their research responsibilities, including updated training for protocol amendments, if applicable
- Financial Disclosure Forms and updates. Members of the Academic Council and Medical Center Line faculty will track their financial disclosures within Stanford's Outside Professional Activities Certification System (OPACS).
- Copies of all official correspondence with the IRB of record, including approval letters, copies of all submissions, and approved template informed consent documents
- Copies of all official correspondence with the Stanford APB, including approval letters, report acceptance letters, and copies of all submissions.
- Copies of all correspondence with the SRC, including approval letters, renewals, and other types of communication
- Manufacturing batch records and documentation of appropriate control of material through the manufacturing process
- Serious adverse event (SAE) case report forms, documenting the subject, date, event, relatedness to investigational agents, follow-up, and outcome, with dates of communication to Oncore/CCTO-Safety and IRB or APB, if applicable
- Log of Deviations, maintained in OnCore
- Laboratory documentation, including copies of CAP and CLIA certificates, State licenses, laboratory director CV and medical license, with laboratory normal values/reference ranges for all labs used in the study

9.6 Printed, dated copy of IRB roster, for each year of the study FDA Oversight

9.6.1 Investigational New Drug (IND) Application Considerations

This study will be conducted under **IND 28621**, held by Sponsor-Investigator Melody Smith, MD. Information from IND 17484 (Sponsor: Crystal Mackall) and IND 018873 (Sponsor: Orca Bio) is incorporated by cross reference.

9.6.1.1 Protocol Amendments

Changes to the general investigational plan; chemistry, manufacturing and controls (CMC) section; and clinical protocol amendments will be submitted to FDA in accordance with 21CFR§312.30 (Protocol Amendments) and 21CFR§312.31 (Information Amendments).

IND annual reports will be submitted to FDA in accordance with 21CFR§312.33 (Annual Reports). The IND annual reports will also be provided to Stanford IRB, APB and the SMC.

10 STATISTICAL CONSIDERATIONS

10.1 Statistical Plan

For this Phase I trial, we will employ the Keyboard design²⁴ find the maximum tolerated dose (MTD). The Keyboard design is a novel Bayesian interval design that is implemented in a simple way similar to the 3+3 design. It is more flexible than the 3+3 design because the decision rules to escalate/de-escalate are driven by the accumulated observations and the observed proportion of dose-limiting toxicities (DLTs) at a given dose level. Furthermore, Keyboard possesses superior operating characteristics because it features a substantially lower risk of overdosing and offers better precision to identify the MTD. These two benefits are due to the fact that as more patients are enrolled at a specific dose, the probability increases that this dose is close to the MTD and it is more likely that the same dose will be recommended for the next cohort to be enrolled.

10.1.1 Statistical Design Parameters

For this trial, the dose-limiting toxicity (DLT) is based on the number of patients who experience either Grade III or IV acute GvHD. The target toxicity rate for the MTD is $\phi = 15\%$, with the acceptable toxicity probability interval ranging from 10% to 20%. This acceptable interval is based on a natural definition of the MTD; namely, the dose at which the risk of DLT is within a pre-specified range. The maximum sample size is 18. We will enroll and treat patients in cohorts of size 3. Patients in the first cohort will be treated at dose level 1. The maximum number of patients who may be treated at any dose level is 12.

10.1.2 Decision Rules for Dose Selection

The Keyboard design decision rules for dose selection are described as follows:

1. To assign a dose to the next cohort of patients, conduct dose escalation/de-escalation according to the rule displayed in Table 6. When using Table 6, please note the following:
 - a. "Eliminate" means that we eliminate the current and higher doses from the trial to prevent treating any future patients at these doses because they are overly toxic.
 - b. When we eliminate a dose, automatically de-escalate the dose to the next lower level. When the lowest dose is eliminated, pause the trial for reevaluation of the dosing strategy. In this case, no dose should be selected as the MTD.
 - c. If none of the actions (i.e., escalation, de-escalation or elimination) is triggered, the current dose will be maintained to treat the next cohort of three patients.
 - d. If the current dose is the lowest dose and the rule indicates dose de-escalation, treat the new patients at the lowest dose unless the number of DLTs reach the elimination boundary, at which time will pause enrollment pending discussion of alternative dosing strategies.
 - e. If the current dose is the highest dose and the rule indicates dose escalation, treat the new patients at the highest dose.
2. Repeat the preceding step until the maximum sample size of 18 is reached or stop the trial if the number of patients treated at the current dose reaches 12 and the decision according to Table 6 is to stay at the current dose.

Table 6 Dose Escalation or de-escalation rule for the Keyboard design

No. of patients treated at current dose	1	2	3	4	5	6	7	8	9	10	11	12
Escalate if # of DLT ≤	0	0	0	0	0	0	0	0	0	0	0	0
Stay at Current Dose if # of DLT ≤	NA	NA	NA	NA	NA	1	1	1	1	1	2	2
Deescalate if # of DLT ≥	1	1	1	1	1	2	2	2	2	2	3	3
Eliminate Current Dose if # of DLT ≥	NA	NA	2	2	2	3	3	3	3	4	4	4

When none of the actions (i.e., escalate, de-escalate or eliminate) is triggered, stay at the current dose for treating the next cohort of patients. “NA” means that a decision cannot be made yet at that number of patients treated with the current dose.

10.1.3 Operating Characteristics

Table 7 shows the operating characteristics of the trial design based on 1000 simulations of the trial using the R/Shiny app “Keyboard” available at <http://www.trialdesign.org>. The operating characteristics show that the design selects the true MTD, if any, with high probability and allocates more patients to the dose levels with the DLT rate closest to the target toxicity rate of 15%.

Table 7 Operating characteristics of the Keyboard design

	Dose -1	Dose 1	Dose 2	Dose 3	Expected Number of Patients	% Early Stopping
Scenario 1						
True DLT rate	0.05	0.15	0.20	0.30		
Selection %	20.8	43.1	25.5	10		0.6
# Pts treated	3.25	7.44	4.32	2.14	17.1	
Scenario 2						
True DLT rate	0.04	0.06	0.15	0.27		
Selection %	3.7	31.2	45.2	19.5		0.4
# Pts treated	1.01	6.37	6.34	3.67	17.4	
Scenario 3						
True DLT rate	0.01	0.10	0.15	0.21		
Selection %	7.2	38.6	31	23.2		0

	Dose -1	Dose 1	Dose 2	Dose 3	Expected Number of Patients	% Early Stopping
# Pts treated	1.78	6.82	5.03	3.62	17.2	
Scenario 4						
True DLT rate	0.02	0.04	0.10	0.15		
Selection %	1.7	19.1	33.9	45.2		0.1
# Pts treated	0.62	5.33	5.62	5.99	17.6	

"% Early Stopping" refers to early stopping due to excessive DLT. Cells are highlighted in each scenario to indicate the dose level at which the target toxicity rate is achieved.

10.1.4 Determination of the MTD

After the trial is completed, select the MTD based on isotonic regression as specified by Yan, Mandrekar and Yuan (2017)²⁴. This computation is implemented by the R/Shiny app "Keyboard", which is available at <http://www.trialdesign.org>. Specifically, select as the MTD the dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there are ties, then select the higher dose level when the isotonic estimate is lower than the target toxicity rate and select the lower dose level when the isotonic estimate is greater than or equal to the target toxicity rate.

Once we determine the MTD, we may expand the cohort by enrolling additional patients to accumulate experience with safety and efficacy.

10.2 Study Endpoints

10.2.1 Primary Endpoints

1. Ability to manufacture donor CD19/CD22-CAR T cells that meet established release specifications will be assessed by the number of product successes – with a goal of 2 of 3 products, 3 of 6 products, 4 of 12 products, or 5 of 18 products.
2. Safety of the donor CD19/CD22-CAR T cells plus the Orca-T graft will be assessed by the incidence of engraftment without Grade III to IV acute GVHD at Day 42 to determine the MTD.

10.2.2 Secondary Endpoints

1. Relapse, disease progression and NRM at 1 year
2. Frequency of secondary graft failure within 1 year
3. PFS and OS at 1 year following Orca-T infusion plus donor CD19/CD22-CAR T cell infusion
4. Greater than Grade 3 infectious disease complication within 1 year

10.3 Interim Analyses

There are no planned interim analyses for the Keyboard dose-finding design.

10.3.1 Feasibility

The failure rate should not exceed 10%. Monitor the number of patients who experience graft failure when either 3, 6, 9, or 12 patients have been treated at a dose level. Pause the study if the number is at least 2 out of 3, at least 3 out of 6, at least 4 out of 12, or at least 5 of 18 patients. These criteria correspond to binomial cumulative probabilities of 2.8%, 1.6%, 2.6%, and 2.8%, respectively, which refer to the chances of observing counts at least as extreme as these under the tolerated incidence rate.

10.3.2 Safety Stopping Rules

The following safety rules that will lead to a pause in the study will be assessed for the number of patients treated at a given dose level.

Episode of Grade 3 or 4 ICANS greater than 7 days: The incidence rate should not exceed 15%. Monitor the number of patients who experience this episode when either 3 or 6 patients have been treated at a dose level. Pause the study if the number is at least 2 out of 3 or at least 3 out of 6. These criteria correspond to binomial cumulative probabilities of 6.1% and 4.7%, respectively, which refer to the chances of observing counts at least as extreme as these under the tolerated incidence rate.

Graft Failure by Day 42: The incidence rate should not exceed 10%. Monitor the number of patients who experience graft failure when either 3, 6, or 9 patients have been treated at a dose level. Pause the study if the number is at least 2 out of 3, at least 3 out of 6, or at least 4 out of 9. These criteria correspond to binomial cumulative probabilities of 2.8%, 1.6%, and 0.8%, respectively, which refer to the chances of observing counts at least as extreme as these under the tolerated incidence rate.

Safety: In an unmodified allogeneic hematopoietic cell transplant with myeloablative conditioning, it is expected that 15% of subjects will not fulfill the criteria for engraftment by Day 100. If 2 of 3, 3 of 6, or 4 of 9 subjects experience primary graft failure, then we will pause the study and evaluate the clinical data.

10.4 Secondary Analysis

10.4.1 Sample Size

The sample size is 12 to 18 patients for the Keyboard design.

10.4.2 Accrual estimates

The recruitment period for this study is expected to be 2 years. Thus, we plan to accrue at least 6 to 9 patients per year.

10.4.2.1 Sample size justification

We allow for the design to test either 12 patients at a single dose level or 3 patients at each of the 4 dose levels for a minimum of 12 patients. In addition, we allow for the design to test 6 additional patients such that all are treated either at a single dose level or 3 at each of 2 dose levels for a maximum of 18 patients. If donor CD19/CD22-CAR T cells cannot be generated for a patient due to a manufacturing failure, then they will be replaced on the study.

10.5 Descriptive Statistics and Exploratory Data Analysis

We plan to investigate the following exploratory analyses, includes analyses related to pharmacokinetics, pharmacodynamics and discovery science:

1. Measure persistence of T_{cons} , T_{regs} , and donor CD19/CD22-CAR T cells in the blood, bone marrow and CSF
2. Investigate donor CD19/CD22CAR T cell properties correlated with CAR T cell efficacy and persistence
3. Evaluate the impact of donor CD19/CD22-CAR T cells on immune reconstitution after Orca-T infusion including its impact on numbers of circulating B cells and bone marrow
4. Assess the impact of the donor CD19/CD22-CAR T cells on the genomic profile of the patients with CAPP-seq compared to historic controls
5. Investigate cytokine signature of the donor CD19/CD22-CAR T cells as compared to CD19/CD22-CAR T cells from autologous sources
6. Assess the impact of the donor CD19/CD22-CAR T cells on the ALL-stem cell and the bone marrow microenvironment
7. Evaluate whether donor CD19/CD22-CAR T cells traffic to GVHD target organs

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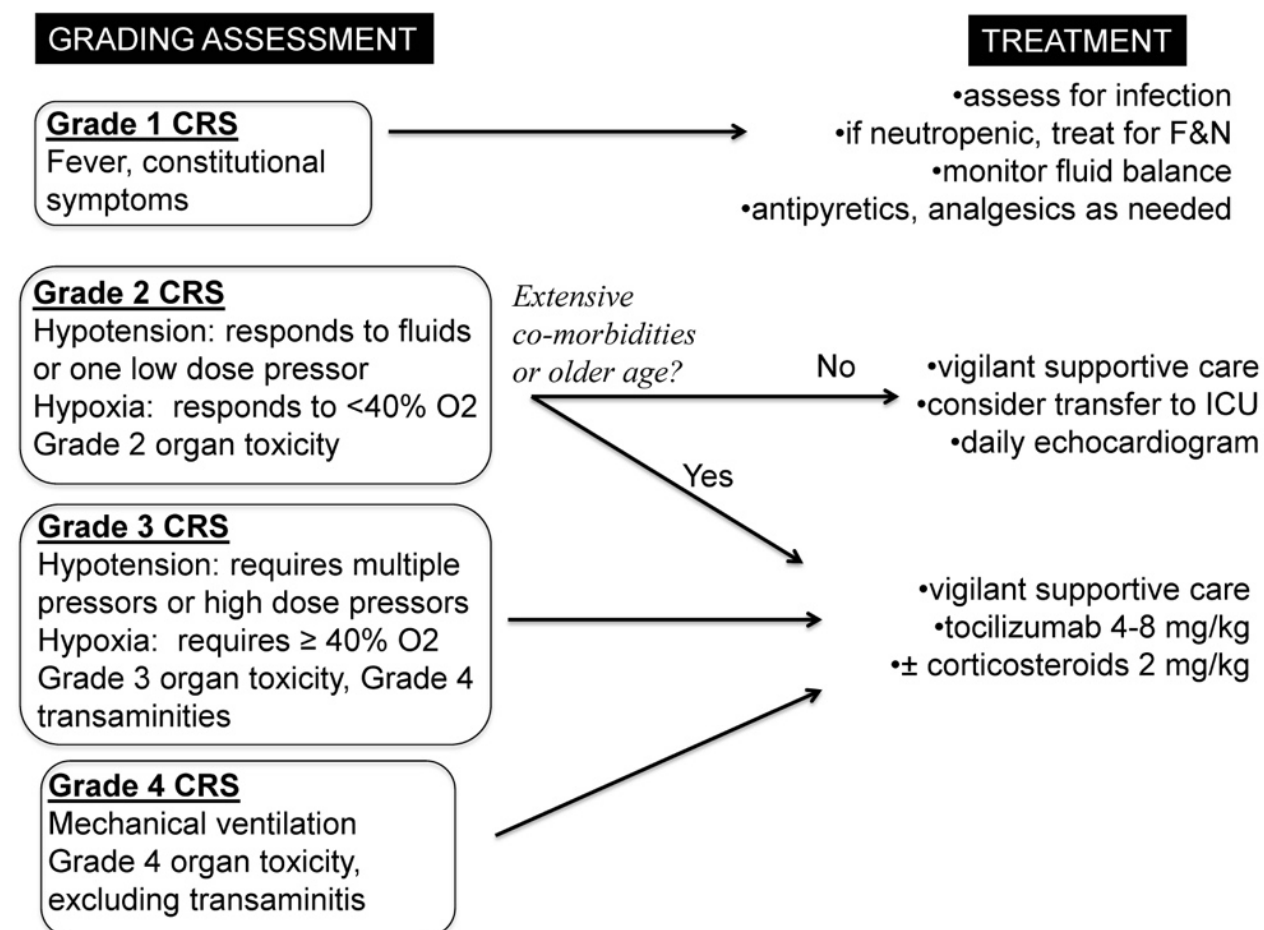
12 APPENDICES

12.1 Appendix A: Performance Status Criteria

PERFORMANCE STATUS CRITERIA <i>Karnofsky and Lansky performance scores are intended to be multiples of 10.</i>					
ECOG (Zubrod)		Karnofsky		Lansky	
Score	Description	Score	Description	Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100%	Normal, no complaints, no evidence of disease.	100%	Fully active, normal.
		90%	Able to carry on normal activity; minor signs of symptoms of disease.	90%	Minor restrictions in physically strenuous activity.
1	Restricted in physically strenuous activity but ambulatory, able to carry out light or sedentary work, e.g., light housework, office work.	80%	Able to carry on normal activity with effort; some signs or symptoms of disease.	80%	Active, but tires more quickly.
		70%	Cares for self, unable to carry on normal activity or do active work.	70%	Both greater restriction of, and less time spent in, play activities.
2	Ambulatory and capable of all self-care but unable to carry out any work activities. Up and about more than 50% of waking hours.	60%	Requires occasional assistance but is able to care for most of own needs.	60%	Up and around, but minimal active play; keeps busy with quieter activities.
		50%	Requires considerable assistance and frequent medical care.	50%	Gets dressed, but lies around much of the day; no active play; able to participate in quiet play and activities.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours	40%	Disabled; requires special care and assistance.	40%	Mostly in bed; participates in quiet activities.
		30%	Severely disabled; hospitalization indicated, although death not imminent.	30%	In bed; needs assistance even for quiet play.
4	Completely disabled. Cannot carry on any self-care. Totally confined to a bed or chair	20%	Very ill; hospitalization necessary; active supportive treatment required.	20%	Often sleeping; play entirely limited to very passive activities.
		10%	Moribund, fatal process progressing rapidly	10%	No play; does not get out of bed
5	Dead	0%	Patient expired	0%	Unresponsive; Dead

12.2 Appendix B: Guidelines for Toxicity Assessment and Management

12.2.1 Guidelines for Grading Suspected Cytokine Release Syndrome according to ASBMT CRS Consensus Grading



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

<u>CRS Parameter</u>	<u>Grade 1</u>	<u>Grade 2</u>	<u>Grade 3</u>	<u>Grade 4</u>
Fever [†]	Temp ≥ 38 °C	Temp ≥ 38 °C	Temp ≥ 38 °C	Temp ≥ 38 °C

With

Hypotension	None	Not requiring vasopressors	Requiring vasopressor	Requiring multiple vasopressors
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			(excluding vasopressin)	(excluding vasopressin)
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And/or§

Hypoxia	None	Requiring low-flow nasal cannula* or blow-by	Requiring high-flow nasal cannula*, facemask, nonrebreather mask, or Venturi mask	Requiring positive pressure (e.g. CPAP, BiPAP, intubation, mechanical ventilation)
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† Fever is defined as temperature $\geq 38^{\circ}$ C not attributable to any other cause. In patients who have CRS then receive anti-pyretics or anti-cytokine therapy such as tocilizumab, anakinra, 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 a temperature of 39.5° C, hypotension requiring one vasopressor and hypoxia requiring low-flow nasal cannula is classified as having Grade 3 CRS.

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

12.2.2 Guidelines for Treating Suspected Cytokine Release Syndrome

Grade 1	<i>Symptoms are not life threatening and require symptomatic treatment only</i> Treatment: ✓ Assess for infection ✓ Treat fever and neutropenia if present, monitor fluid balance, antipyretics, analgesics as needed.
Grade 2	<i>Symptoms require and respond to moderate intervention</i> Treatment: ✓ As above for Grade 1 ✓ Monitor organ function closely (incl. cardiac, respiratory, renal, neurologic, liver) ✓ Consider tocilizumab with or without corticosteroids
Grade 3 or Grade 2 with co-morbidity	<i>Symptoms require and respond to aggressive intervention</i> Treatment: ✓ As above for Grade 2 ✓ Tocilizumab with or without corticosteroids ✓ Consider anakinra ✓ Consider vasopressors, oxygen supplementation
Grade 4	<i>Life-threatening symptoms</i> Treatment: ✓ As above ✓ Positive pressure airway assistance or Mechanical ventilation ✓ Multiple vasopressors or high dose vasopressors

High dose vasopressor doses shown in **Table 2**.

Tocilizumab is administered at a dose of 4-8 mg/kg infused over 1 hour (not to exceed 800 mg).

Table 2. High-Dose Vasopressors (all doses are required for ≥ 3 hours)

Norepinephrine monotherapy	≥ 0.2 mcg/kg/min
Dopamine monotherapy	≥ 10 mcg/kg/min
Phenylephrine monotherapy	≥ 200 mcg/min
Epinephrine monotherapy	≥ 0.1 mcg/kg/min
If on vasopressin	¹ Vasopressin + NE equivalent of ≥ 0.1mcg/kg/min
If on combination vasopressors (not vasopressin)	¹ Norepinephrine equivalent of ≥ 20 mcg/kg/min

¹VASST Trial Vasopressor Equivalent Equation:

Norepinephrine equivalent dose = [norepinephrine (mcg/min)] + [dopamine (mcg/kg/min) ÷ 2] + [epinephrine (mcg/min)] + [phenylephrine (mcg/min) ÷ 10]

12.2.3 Encephalopathy assessment tools for grading Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS)

12.2.3.1 Immune Effector Cell-associated Encephalopathy (ICE) Assessment

Directions: Answer whether each task was performed correctly (Not Done/Yes/No). If you answer YES put 1 in the Score column; If you answer NO (or Not Done) put 0 in the Score column.

Tasks	Performed correctly?	Score
Orientation		
1. What is the current year?		0
2. What is the current month?		0
3. What is the current city?		0
4. What hospital are you in?		0
Naming		
5. Name this object (<i>point to an object in the room</i>)		0
6. Name this object (<i>point to an object in the room</i>)		0
7. Name this object (<i>point to an object in the room</i>)		0
Following commands		
8. Show me (insert object, e.g. 2 fingers) or Close your eyes and stick out your tongue.		0
Writing		
9. Write a simple sentence (<i>provide paper and pencil</i>)		0
Attention		
10. Count backwards from 100 in 10's.		0
Total Score		

Scoring ICE

No impairment: Score 10

Grade 1 ICANS: Score 7-9

Grade 2 ICANS: Score 3-6

Grade 3 ICANS: Score 0-2

Grade 4 ICANS: Score 0 due to subject unarousable and unable to perform ICE assessment.

12.2.3.2 ASBMT Immune effector Cell-Associated Neurotoxicity Syndrome (ICANS) Consensus Grading for Adults

Neurotoxicity Domain	Grade 1	Grade 2	Grade 3	Grade 4
ICE Score[^]	7-9	3-6	0-2	0 (patient is unarousable and unable to perform ICE)
Depressed level of consciousness[‡]	Awakens spontaneously	Awakens to voice	Awakens only to tactile stimulus	Patient is unarousable or requires vigorous or repetitive tactile stimuli to arouse. Stupor or coma
Seizure	N/A	N/A	Any clinical seizure focal or generalized that resolves rapidly; or Non-convulsive seizures on EEG that resolve with intervention	Life-threatening prolonged seizure (> 5 min); or Repetitive clinical or electrical seizures without return to baseline in between.
Motor findings[§]	N/A	N/A	N/A	Deep focal motor weakness such as hemiparesis or paraparesis
Raised 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. For example, a patient with an ICE score of 3 who has a generalized seizure is classified as having a Grade 3 ICANS.

[^]A patient with an ICE score of 0 may be classified as having Grade 3 ICANS if the patient is awake with global aphasia. But a patient with an ICE score of 0 may be classified as having a Grade 4 ICANS if the patient is 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 graded according to CTCAE v5.0 but they do not influence ICANS grading.

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

ICE: Immune effector Cell-associated Encephalopathy; **ICP:** Intracranial pressure; **EEG:** electroencephalogram

12.3.5 Neurotoxicity Assessment and Management Guidance

Neurotoxicity Grading assessment (CTCAEv5.0)	Recommended Treatment *	Evaluation
Grade 1: (consider ICANS score) Examples include: - Somnolence-mild drowsiness or sleepiness - Confusion-mild disorientation - Encephalopathy- mild limiting of ADL - Dysphasia-not impairing ability to communicate - Brief partial seizure; no loss of consciousness	☐ Notify MD ☐ Ensure IV access ☐ Consider increasing dose of Keppra, consider alternative antiepileptics ☐ Neurologic Consult ☐ Institute Aspiration precautions ☐ Consider tocilizumab in NS 8 mg/kg (if ≥ 30 kg) or 12 mg/kg (if < 30 kg), IV if concurrent CRS (grade 2 or 3)	Neuro checks Q 2-4 h, wake for neuro checks Neurologic Consult EEG or MRI of brain Review for causes of confusion, i.e. polypharmacy, and avoid CNS depressive meds Additional work up as clinically indicated.
Grade 2: (consider ICANS score) Examples include: - Somnolence-moderate, limiting instrumental ADL - Confusion-moderate disorientation, limiting instrumental ADL - Encephalopathy- limiting instrumental ADL - Dysphasia-moderate impairing ability to communicate spontaneously - Brief generalized seizure	Include those above for Grade 1 ☐ Vigilant supportive care ☐ Elevate HOB $> 30^\circ$ ☐ Consider ICU transfer if associated with grade ≥ 2 CRS ☐ Increase levetiracetam dose from 1 GMBID ☐ Consider increasing Keppra dose to 1 GM IV BID ☐ Consider Steroids: Dexamethasone 10mg q 6-12 hours (methylprednisolone 1 mg/kg IV Q12h; OR dexamethasone 0.1 mg/kg	Brain MRI (with or without contrast) Evaluation of CSF Consider EEG as clinically indicated Cardiac telemetry and continuous pulse oximetry VS Q 2-4h, notify MD immediately for bradycardia, hypertension, or irregular respirations. Q4 hour neurological checks (wake the subject)

	IV Q6h) or ICANS without CRS	
Grade 3: Examples include: • Somnolence-obtundation or stupor • Confusion-severe disorientation, limiting self-care ADL • Encephalopathy- limiting self-care ADL • Dysphasia-severe receptive or expressive characteristics, impairing ability to read, write or communicate intelligibly • Multiple seizures despite medical intervention • Weakness limiting self-care ADL; disabling • Complete bowel / bladder incontinence	Include those above for Grade 1 ☐ Tocilizumab 8 mg/kg IV over 1 hour (not to exceed 800 mg), q 4-6 hours if symptoms have not stabilized or improved within 12-24 hours ☐ Corticosteroids: (methylprednisolone 30 mg/kg IV Q24h X 3 for ICANS without CRS ☐ Consider use of anakinra ☐ ICU transfer, if not previously done ☐ Consider IT hydrocortisone ☐ Assess need for airway protection	As above for Grade 1 & 2 Evaluate for papilledema and treat elevated ICP if present
Grade 4: Examples include: • Life-threatening consequences • Urgent intervention indicated • Mechanical ventilation • Life-threatening; prolonged repetitive seizures	As above plus: ☐ Airway support and/or Mechanical ventilation, and all other supportive care measures ☐ Give high dose steroids: methylprednisolone 1 g or 30 mg/kg, daily x3 (MAX 1000 mg) with taper ☐ Mechanical ventilation and other supportive measures	As above plus: Evaluate for status epilepticus and treat accordingly

* **Please NOTE:** these are only recommendations and actual physician's plan of care must consider patient conditions, comorbidities, neurologist recommendations, etc.

12.3 Appendix C: Assessment and Management of Acute GVHD

Grading of Individual Organ Systems

Organ	Stage	Description
<u>Skin</u>	+1	Maculo-papular eruption over < 25% of body area
	+2	M-P eruption over > 25 to 50% of body area
	+3	Generalized erythroderma
	+4	Generalized erythroderma with bullous formation and often with desquamation
<u>Liver</u>	+1	Bilirubin 2.0 to 3.0 mg/dL; SGOT 150 to 750IU
	+2	Bilirubin 3.1 to 6.0 mg/dL
	+3	Bilirubin 6.1 to 15.0 mg/dL
	+4	Bilirubin >15.0 mg/dL
<u>Gut</u>	+1	Diarrhea > 30 mL/kg or > 500 mL/day
	+2	Diarrhea > 60 mL/kg or > 1000 mL/day
	+3	Diarrhea > 90 mL/kg or > 1500 mL/day
	+4	Diarrhea > 90 mL/kg or > 2000 mL/day; or severe abdominal pain with or without ileus

Overall Grade

Grade	Skin	Liver		Gut	ECOG Performance
I	+1 to +2	0		0	0
II	+1 to +3	+1	and/or	+1	0 to 1
III	+2 to +3	+2 to +4	and/or	+2 to +3	2 to 3
IV	+2 to +4	+2 to +4	and/or	+2 to +4	3 to 4

12.5 Appendix D: Response Criteria

12.5.1 NCCN Guidelines Version 4. 2021 Acute Lymphoblastic Leukemia



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RESPONSE ASSESSMENT

Response Criteria for Blood and Bone Marrow:

- CR
 - No circulating lymphoblasts or extramedullary disease
 - ◊ No lymphadenopathy, splenomegaly, skin/gum infiltration/testicular mass/CNS involvement
 - Trilineage hematopoiesis (TLH) and <5% blasts
 - Absolute neutrophil count (ANC) >1000/microL
 - Platelets >100,000/microL
 - No recurrence for 4 weeks
- CR with incomplete blood count recovery (CRi)
 - Meets all criteria for CR except platelet count or ANC
- Overall response rate (ORR = CR + CRi)
- **NOTE:** MRD assessment is not included in morphologic assessment and should be obtained ([see ALL-F](#))
- Refractory disease
 - Failure to achieve CR at the end of induction
- Progressive disease (PD)
 - Increase of at least 25% in the absolute number of circulating or bone marrow blasts or development of extramedullary disease
- Relapsed disease
 - Reappearance of blasts in the blood or bone marrow (>5%) or in any extramedullary site after a CR

Response Criteria for CNS Disease:

- CNS remission: Achievement of CNS-1 status ([see ALL-B](#)) in a patient with CNS-2 or CNS-3 status at diagnosis.
- CNS relapse: New development of CNS-3 status or clinical signs of CNS leukemia such as facial nerve palsy, brain/eye involvement, or hypothalamic syndrome without another explanation.

Response Criteria for Lymphomatous Extramedullary Disease:

- CT of neck/chest/abdomen/pelvis with IV contrast and PET/CT should be performed to assess response for extramedullary disease.
- CR: Complete resolution of lymphomatous enlargement by CT. For patients with a previous positive PET scan, a post-treatment residual mass of any size is considered a CR as long as it is PET negative.
- PR: >50% decrease in the sum of the product of the greatest perpendicular diameters (SPD) of the mediastinal enlargement. For patients with a previous positive PET scan, post-treatment PET must be positive in at least one previously involved site.
- PD: >25% increase in the SPD of the mediastinal enlargement. For patients with a previous positive PET scan, post-treatment PET must be positive in at least one previously involved site.
- No Response (NR): Failure to qualify for PR or PD.
- Relapse: Recurrence of mediastinal enlargement after achieving CR. For patients with a previous positive PET scan, post-treatment PET must be positive in at least one previously involved site.

Note: All recommendations are category 2A unless otherwise indicated.

Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.



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MINIMAL/MEASURABLE RESIDUAL DISEASE ASSESSMENT

- The preferred sample for MRD assessment is the first small volume (of up to 3 mL) pull of the bone marrow aspirate, if feasible.
- MRD in ALL refers to the presence of leukemic cells below the threshold of detection by conventional morphologic methods. Patients who achieved a CR by morphologic assessment alone can potentially harbor a large number of leukemic cells in the bone marrow.
- MRD is an essential component of patient evaluation over the course of sequential therapy. If validated MRD assessment technology with appropriate sensitivity (at least 10^{-4}) is not available locally, there are commercially available tests.
- Studies in both children and adults with ALL have demonstrated the strong correlation between MRD and risks for relapse, as well as the prognostic significance of MRD measurements during and after initial induction therapy.¹
- The most frequently employed methods for MRD assessment include at least 6-color flow cytometry assays^{2,3} specifically designed to detect abnormal MRD immunophenotypes, real-time quantitative polymerase chain reaction (RQ-PCR) assays to detect fusion genes (eg, *BCR-ABL1*), and NGS-based assays, to detect clonal rearrangements in immunoglobulin (Ig) heavy chain genes and/or T-cell receptor (TCR) genes.
- Current 6-color flow cytometry can detect leukemic cells at a sensitivity threshold of $<1 \times 10^{-4}$ ($<0.01\%$) bone marrow mononuclear cells (MNCs).^{2,3} PCR/NGS methods can detect leukemic cells at a sensitivity threshold of $<1 \times 10^{-6}$ ($<0.0001\%$) bone MNCs.^{4,5} The concordance rate for detecting MRD between these methods is generally high. Methods not achieving these sensitivity levels are not suitable.
 - For flow cytometric analysis of MRD, notify lab performing the MRD assessment if immunotherapy (such as rituximab, blinatumomab, inotuzumab ozogamicin, or tisagenlecleucel) has been used.
 - Timing of MRD assessment:
 - ◇ Upon completion of initial induction.
 - ◇ Additional time points should be guided by the regimen used.
 - ◇ Serial monitoring frequency may be increased in patients with molecular relapse or persistent low-level disease burden.
 - ◇ For some techniques, a baseline sample may be needed or helpful for the MRD assessment to be valid.

¹ Berry DA, Zhou S, Higley H, et al. Association of minimal residual disease with clinical outcome in pediatric and adult lymphoblastic leukemia. *JAMA Oncol* 2017;3:e170580.

² Gaipa G, Cazzaniga G, Valsecchi MG, et al. Time point-dependent concordance of flow cytometry and real-time quantitative polymerase chain reaction for minimal residual disease detection in childhood acute lymphoblastic leukemia. *Haematologica* 2012;97(10):1582-1593.

³ Denys B, van der Sluijs-Gelling AJ, Homburg C, et al. Improved flow cytometric detection of minimal residual disease in childhood acute lymphoblastic leukemia. *Leukemia* 2013;27:635-641.

⁴ Bruggemann M, Schrauder A, Raff T, et al. Standardized MRD quantification in European ALL trials: proceedings of the Second International Symposium on MRD assessment in Kiel, Germany, 18-20 September 2008. *Leukemia* 2010;24:521-535.

⁵ Campana D. Minimal residual disease in acute lymphoblastic leukemia. *Hematology Am Soc Hematol Educ Program* 2010;2010:7-12.

Note: All recommendations are category 2A unless otherwise indicated.

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NCCN Categories of Evidence and Consensus	
Category 1	Based upon high-level evidence, there is uniform NCCN consensus that the intervention is appropriate.
Category 2A	Based upon lower-level evidence, there is uniform NCCN consensus that the intervention is appropriate.
Category 2B	Based upon lower-level evidence, there is NCCN consensus that the intervention is appropriate.
Category 3	Based upon any level of evidence, there is major NCCN disagreement that the intervention is appropriate.

All recommendations are category 2A unless otherwise indicated.

NCCN Categories of Preference	
Preferred intervention	Interventions that are based on superior efficacy, safety, and evidence; and, when appropriate, affordability.
Other recommended intervention	Other interventions that may be somewhat less efficacious, more toxic, or based on less mature data; or significantly less affordable for similar outcomes.
Useful in certain circumstances	Other interventions that may be used for selected patient populations (defined with recommendation).

All recommendations are considered appropriate.

12.6 Appendix E: Correlative Studies Table

Correlative Sample Collection Matrix																						
Volume (mL)	Type	Purpose	Unmobilized Apheresis	Mobilized Apheresis	Baseline (prior to LD chemo)	D0 (± 2 d)	D3 (± 2 d)	D7 (± 2 d)	D10 (± 2 d)	D14 (± 2 d)	D21 (± 4 d)	D30 (± 4 d)	D42 (± 1 w)	M2 (± 2 w)	M3 (± 2 w)	M4 (± 2 w)	M5 (± 2 w)	M6 (± 1 m)	M9 (± 1 m)	M12 (± 2 m)	At Progression (± 1 m)	Onset of GVHD (± 2 w)
Apheresis Product																						
5	Collected by Stanford		X ¹	X ¹																		
CAR T cell Product																						
5	Collected by Stanford	Flow panel (CAR-FACS, T _{reg} , T _{con})				X																
5	Collected by Stanford	scRNA-Seq, TCR sequencing, cite-seq ^{2,8}				X																
5	Collected by Stanford	ATAC-Seq				X																
Peripheral blood mononuclear cells (PBMCs)																						
10	Green top (Heparin)	Flow panel (CAR-FACS, T _{reg} , T _{con}) ³			X			X ¹¹	X ¹¹			X		X	X			X	X	X	X	X
10	Lavender top (EDTA)	scRNA-Seq, TCR sequencing, cite-seq ^{2,3}			X			X ¹⁰	X ¹⁰			X ⁹		X ¹⁰	X ¹⁰			X ¹⁰	X ¹⁰	X ¹⁰	X ¹⁰	X ⁹
10	Green top (Heparin)	CyTOF (CAR-FACS, Treg, Tcon) ³			X			X	X			X		X	X			X	X	X	X	X
10	Lavender top (EDTA)	CAR qRT-PCR ^{2,3}						X ¹²	X ¹²			X		X	X ²			X ²	X	X ²	X ²	X ²
5	Lavender top (EDTA)	ATAC-Seq ³			X							X			X			X		X	X	X
10	Green top (Heparin)	Sample banking				X	X	X	X	X	X	X		X	X	X	X	X	X	X	X	X
Plasma																						
4	Lavender top (EDTA)	Cytokines - Luminex (HIMC) ³				X	X	X ⁷	X	X ⁷	X	X ⁷										
4	Lavender top (EDTA)	MRD - BALL				X		X ⁷	X ⁷	X	X ⁷			X ⁷	X ⁷			X ⁷	X ⁷	X ⁷		
4	Lavender top (EDTA)	Plasma metabolomics ³			X ⁷	X	X	X ⁷	X	X ⁷	X	X ⁷		X ⁷	X ⁷			X ⁷	X ⁷	X ⁷	X ⁷	X ⁷
Fecal Samples																						
	Collected by Stanford	Intestinal microbiome analysis ^{3,6}			X	X		X		X	X			X	X			X	X	X	X	X
Bone Marrow Aspirate																						
2	Green top (Heparin)	Flow panel (CAR-FACS) ³			X ⁵								X		X			X		X	X	
5	Green top (Heparin)	CyTOF ³			X ⁵								X		X			X		X	X	
5	Lavender top (EDTA)	scRNA-Seq, TCR sequencing ³			X ⁵								X		X			X		X	X	
5	Lavender top (EDTA)	ATAC-Seq ³			X ⁵								X		X			X		X	X	
2	Green top (Heparin)	Sample banking			X ⁵								X		X			X		X	X	
Lumbar Puncture/CSF																						
1	Plastic Falcon tube				X ⁵							X ^{4,5}									X ^{4,5}	
GVHD-target organs																						
	Two samples of biopsy specimen collected in RPMI	scRNA-Seq																				X
	Two samples of biopsy specimen	Potential assessment with formalin-fixed, paraffin-embedded (FFPE)																				X
Notes																						
1	Small volume should be taken from apheresis collection																					
2	Sample collected used for both CAR qRT-PCR and TCR sequencing																					
3	Stop further collection if CAR is undetectable.																					
4	Collect at any time of neurotoxicity and at Day 28 (for subjects with CNS at baseline), at discretion of investigator																					
5	If clinically indicated, then these samples will be obtained																					
6	Samples will be submitted for 16S shotgun sequencing, metagenomic shotgun sequencing, and fecal metabolomics.																					
7	These studies will be collected from plasma obtained from PBMC samples at the respective timepoints.																					
8	For sc-RNA seq of the CAR product, will assess both the CAR positive and CAR negative fraction.																					
9	Sort PBMCs prior to sc-RNA seq.																					
10	scRNA-seq will be performed on bulk PBMCs.																					
11	Flow panel D7 and D14 timepoints																					
12	CAR qRT-PCR D7 and D14 timepoints																					
Samples	CAR Product	Peripheral Blood	Bone Marrow																			
Real Time	- N/A	- FACS - scRNA-Seq, TCR sequencing, cite-seq - GVHD pathology	- scRNA seq, TCR sequencing																			
Batched	- All assays will be banked	- CAR qRT-PCR - CyTOF - ATAC-Seq	- FACS - qRT-PCR - CyTOF - ATAC-Seq																			

12.7 Appendix F: Monitoring Gene Therapy Trials: Replication Competent Lentivirus (RCL)

Subject Testing for RCL PCR

Subject blood samples will be obtained at the following time points and banked for future testing:

1. Prior to cell infusion
2. 3 months ($\pm$ 1 month)
3. 6 months ($\pm$ 1 month)
4. 12 months ($\pm$ 2 months)

All samples are scheduled from the most recent cell infusion, and should proceed even after disease progression or proceeds to alternative therapy; the only exception is if the subject proceeds to stem cell transplant with myeloablative preparative regimen. RCL testing of archived samples will occur if events are considered concerning for long-term new or worsening suspected adverse reaction or for events described in **Section 8**.

Procedures:

Samples will be collected from subjects returning to clinic or, at investigator discretion, may be collected by a local medical provider. Samples will be collected and stored according to CCT SOPs 'RCL Monitoring' and 'Remote RCL/RCR Monitoring'.

Document Retention

1. RCL Reports from Indiana University will be sent to the Sponsor Investigator or designated representative; who will distribute the results. Groups requesting RCL results notification include:
 - a. Principal Investigator and co-investigators
 - b. IND Sponsor, Crystal Mackall
 - c. CCT-Clinical Research Operations & Procedures (CCT-CROP) [regulatory support]
 - d. CCT Manufacturing team
2. Hard copies will be retained in the subject's research record or other suitable study file in a locked file cabinet.

12.8 Appendix G: Draft Letter and Questionnaire to Subjects for Long Term Follow-up for Delayed Adverse Events

[Date]

[Name and address]

Dear [Subject name],

You have participated in a clinical research study that requires that the study doctors and nurses monitor your health for 15 years. In addition to the annual visits you will be attending, **we would like for you to report certain events listed below to your study doctor or nurse if they occur:**

1. Your doctor tells you that you have been diagnosed with any new type of cancer, including blood disorders such as leukemia or lymphoma (this would be separate from your cancer diagnosis).
2. You develop loss of feeling in any part of your body, especially hands and feet; you develop a loss of control of any body part (arms, legs, etc.); you have a seizure; you experience memory loss. In addition, if you experience a worsening of any of the symptoms listed, please contact your study nurse or doctor. These types of symptoms are called neurological disorders. If your primary doctor or specialist tells you that you have developed neurological symptoms, contact your study doctor or nurse.
3. You develop arthritis or autoimmune disease, or worsening of any previously experienced arthritis or autoimmune disease which you were experiencing prior to participation in the study. If you are experiencing symptoms of arthritis or have been told by your doctor that you have an autoimmune disease, contact your study doctor or nurse.

Please complete the attached questionnaire and return it in the Fed-Ex envelope to the study coordinator.

If you experience any of the events listed above during the upcoming year, please contact your study physician or the study nurse listed below as soon as you can. They may ask you questions about your health and will record your symptoms/disease and then monitor your health if they decide that it is necessary. When you call, please mention that you participated in a gene therapy clinical trial at the <put your institution here>. Your subject identification number under this protocol is (#XXX).

Study Coordinator:

Name

Address

Phone

Email

If you have any questions about this letter or the follow up procedures for the study itself, please do not hesitate to contact the above study nurse.

Thank you for your continued participation in our clinical research study. Best regards,

[Study coordinator]

Questionnaire to Subjects for Long Term Follow-up for Delayed Adverse Events

Subject Identification [put subject study number here]

Within the past year, have you:

1. Had any problems with your health? ☐ YES ☐ NO

If Yes, please explain: _____

2. Required any hospitalizations? ☐ YES ☐ NO

If Yes, please describe when and the reason: _____

3. Seen any healthcare provider? ☐ YES ☐ NO

If Yes, please describe when and the reason: _____

4. Started on any new medications? ☐ YES ☐ NO

If Yes, please list: _____

5. Developed any new conditions or illnesses? ☐ YES ☐ NO

If Yes, please describe: _____

Please share any other new health concerns or problems: _____

When you have completed this questionnaire, please return it to:

Study Coordinator:

Name

Address

Fax

Phone

Email

A pre-addressed stamped envelope has been enclosed for your convenience, if you choose to mail this questionnaire. We will also accept faxed or e-mailed completed questionnaires as well.

Thank you very much for your participation.

12.9 Appendix H: Physician (Local Medical Provider) Letter

[Date]

[Name and Address]

Dear [Physician name],

Your subject [Subject name] has participated in a clinical research study that requires 15 year monitoring for adverse events. To aid in reporting adverse events that are possible related to the clinical research study, we are asking the subjects on our research study to designate a primary care or infectious disease physician that may help in the monitoring and reporting of adverse events. Your subject has designated you. **If upon any of your visits with your subject, any of the following events are reported or discovered, please contact the study nurse or physician as soon as possible:**

1. New malignancies
2. New incidence or exacerbation of a pre-existing neurologic disorder
3. New incidence or exacerbation of a prior rheumatologic or other autoimmune disorder
4. New incidence of a hematologic disorder.

If your subject experiences any of these events, please contact the study coordinator below as soon as you can so that they can record the event and then monitor your subject's health if necessary. When you call, please mention that the subject has participated in a gene therapy clinical trial in the < designate location, institution and investigator> .

Study Coordinator

Name

Address

Phone

Email

If you have any questions about this letter or the study itself, then please do not hesitate to contact the above study nurse.

Thank you for your support in helping us to monitor for delayed adverse events.

Best regards,

12.10 Appendix I: NCCN Guidelines Version 1.2022 Acute Lymphoblastic Leukemia – Cytogenetic Risk Groups for B-ALL



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CYTOGENETIC RISK GROUPS FOR B-ALL

RISK GROUPS	CYTOGENETICS
Good risk	- Hyperdiploidy (51–65 chromosomes) - Cases with trisomy of chromosomes 4, 10, and 17 appear to have the most favorable outcome - t(12;21)(p13;q22): <i>ETV6-RUNX1</i>^a
Poor risk	- Hypodiploidy^{b,c} (<44 chromosomes) <i>KMT2A</i> rearranged (t[4;11] or others) - t(v,14q32)/IgH - t(9;22)(q34;q11.2): <i>BCR-ABL1</i> (defined as high risk in the pre-TKI era) - Complex karyotype (5 or more chromosomal abnormalities) <i>BCR-ABL1</i>-like (Ph-like) ALL - JAK-STAT (<i>CRLF2</i>;^{c,d} <i>EPOR</i>, <i>JAK1/2/3</i>, <i>TYK2</i>, mutations of <i>SH2B3</i>, <i>IL7R</i>, <i>JAK1/2/3</i>) - ABL class (rearrangements of <i>ABL1</i>, <i>ABL2</i>, <i>PDGFRA</i>, <i>PDGFRB</i>, <i>FGFR</i>) - Other (<i>NTRK</i>, <i>FLT3</i>, <i>LYN</i>, <i>PTK2Br</i>) - Intrachromosomal amplification of chromosome 21 (iAMP21) - t(17;19): <i>TCF3-HLF</i> fusion - Alterations of <i>IKZF1</i>^{e,f}

^a The translocation t(12;21)(p13;q22) is typically cryptic by karyotyping and requires FISH or PCR to identify.

^b There are other results that are not less than 44 chromosomes that may be equivalent to hypodiploidy and have the same implications. It is important to distinguish true hypodiploidy from masked hypodiploidy, which results from the doubling of hypodiploid clones. Carroll AJ, Shago M, Mikhail FM, et al. Masked hypodiploidy: Hypodiploid acute lymphoblastic leukemia (ALL) mimicking hyperdiploid ALL in children: A report from the Children's Oncology Group. *Cancer Genet* 2019;238:62-68.

^c Alternatively defined as DNA index less than protocol-defined threshold or other clear evidence of hypodiploid clone. Hypodiploid ALL is also often associated with *TP53* loss of function mutations and Li-Fraumeni syndrome.

^d Jain N, Roberts KG, Jabbour E, et al. Ph-like acute lymphoblastic leukemia: a high-risk subtype in adults. *Blood* 2017;129:572-581; Roberts KG, Li Y, Payne-Turner D, et al. Targetable kinase-activation lesions in Ph-like acute lymphoblastic leukemia. *N Engl J Med* 2014;371:1005-1015.

^e Mullighan CG, Su X, Zhang J, et al. Deletion of *IKZF1* and prognosis in acute lymphoblastic leukemia. *N Engl J Med* 2009;360:470-480; Stanulla M, Dagdan E, Zaliouva M, et al. *IKZF1*plus defines a new minimal residual disease-dependent very-poor prognostic profile in pediatric B-cell precursor acute lymphoblastic leukemia. *J Clin Oncol* 2018;36:1240-1249.

^f Emerging evidence suggests *DUX4r* ALL is favorable. Additionally in cases of *DUX4r*, *IKZF1* alterations do not confer poor prognosis.

Note: All recommendations are category 2A unless otherwise indicated.
Clinical Trials: NCCN believes that the best management of any patient with cancer is in a clinical trial. Participation in clinical trials is especially encouraged.

ALL-A
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