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Safety and Efficacy of TCRαβ+/CD19+ Depleted Allogeneic Hematopoietic Stem Cell Transplantation for Malignant and Non-malignant Disorders in Children and Adolescent/Young Adult Patients

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ABSTRACT

The purpose of this study is to investigate the safety and efficacy of TCRαβ+/CD19+ depleted allogeneic hematopoietic stem cell transplant (HSCT) for malignant and non-malignant disorders in children and adolescent/young adult patients. This protocol will be utilized to provide patients who lack an HLA-genotypically matched related donor (MRD) with an option to undergo HSCT. We hypothesize that TCRαβ+/CD19+ depleted hematopoietic stem cell products will be safe, effective, and limit acute grades II-IV graft versus host disease (GVHD). TCRαβ+/CD19+ depleted hematopoietic stem cells will be infused with or without a preparative regimen clinically prescribed based on our center's standard-of-care. This therapy serves as an alternative to no HSCT or HSCT with a high risk of acute GVHD, which is known to be associated with significant morbidity and mortality. TCRαβ+/CD19+ depletion will be performed on donor hematopoietic stem cell products using the CliniMACS® immunomagnetic selection device (Miltenyi Biotec). Previous reports have shown this approach to be safe with results equivalent to matched unrelated donor (URD) transplant outcomes.

I. PURPOSE OF STUDY

Acute graft versus host disease (GVHD) remains a significant cause of morbidity and mortality and is the biggest barrier to successful allogeneic hematopoietic cell transplantation (HSCT) outcomes. Improved methods of acute GVHD prevention are needed. TCRαβ+/CD19+ depletion of allogeneic hematopoietic stem cell products offers an opportunity to limit the risk of acute GVHD by removing TCRαβ+ T cells and CD19+ B cells which participate in acute GVHD initiation and perpetuation. The purpose of this study is to investigate the safety and efficacy of TCRαβ+/CD19+ depleted allogeneic hematopoietic stem cell transplant (HSCT) for malignant and non-malignant disorders in children and adolescent/young adult patients using the CliniMACS® immunomagnetic selection device (Miltenyi Biotec).

Hypothesis

TCRαβ+/CD19+ depleted hematopoietic stem cell products will be safe, effective, and limit acute GVHD.

Primary Objective

Assess the safety of infusion of TCRαβ+/CD19+ depleted donor stem cell products

Secondary Objectives

Assess engraftment, acute and chronic GVHD incidence, and 1 year GVHD-free survival

II. SIGNIFICANCE OF STUDY IN RELATION TO HUMAN HEALTH

Allogeneic hematopoietic cell transplantation (HSCT) has been used extensively to treat patients with malignancies, hematologic disorders, and primary immune deficiencies.[1] Ideally, allogeneic HSCT is performed using an HLA-matched related or unrelated donor. However, only approximately 25% of patients will have an HLA-identical sibling, and the chances of finding an HLA-matched unrelated donor vary from 60 to 70% for Caucasian patients to less than 10% for ethnic minorities.[2] The lack of a suitable donor means that some patients are not offered the option of

allogeneic HSCT, or must proceed using mismatched donors which greatly increases the risk of GVHD and death.

Haploidentical transplant offers an attractive alternative because almost all patients will have a haploidentical family member who could potentially serve as the stem cell donor. This type of transplant presents the challenge of a very high risk of GVHD. Mature donor T-cells are responsible for GVHD.[3,4] Methods to T-cell deplete the graft or suppress the donor T cells have been developed to prevent GVHD after HLA-haploidentical HSCT.[5-8] Isolation and infusion of purified CD34+ cells can prevent severe GVHD in haplo-HSCT patients.[9,10] However, this can result in an increased risk of infections early after HSCT. In order to reduce this risk, alloreactive TCRαβ+ T-cells and antigen-presenting CD19+ B-cells can be *selectively* removed from the graft.[11] This approach avoids GVHD but allows the retention of TCRγδ+ T cells and NK cells in the graft to provide early innate immunity. The activation of γδ T-cell activation is not regulated by MHC molecules, which makes them less likely to cause GVHD. TCRγδ+ T cells are known to participate in immunity against cytomegalovirus (CMV) reactivation and disease following HSCT,[12] and expansion of CMV-reactive TCRγδ+ T-cells can be detected in patients who have been treated with kidney or lung transplantation.[13,14] TCR γδ+ T-cells and NK cells may additionally offer protection against other infections and malignancy relapse.[15-18] Importantly, pediatric studies have demonstrated that relapse rates are similar to rates in patients undergoing sibling or unrelated donor transplant while GVHD-free/relapse-free survival is better.[19-21]

Access to TCRαβ+/CD19+ depleted allogeneic HSCT is important as it will expand the access to transplant tremendously: essentially 100% of patients needing HSCT will have a donor option. TCRαβ+/CD19+ depleted HSCT can also be offered to children for whom rapidly progressive or unstable disease does not allow the time to identify a suitable matched URD. Additionally, some patients may have organ dysfunction prior to transplant that prevents giving common GVHD prophylaxis such as cyclosporine or tacrolimus. TCRαβ+/CD19+ depleted HSCT would allow for HSCT without any post-HSCT immune suppression in these patients. Similarly, rare subgroups of pediatric disorders have underlying defects in DNA damage repair pathways, telomere biology, or epithelial cell propensity for abnormal inflammatory responses and cell death (e.g. Fanconi anemia, dyskeratosis congenita, XIAP Deficiency, etc). GVHD can be associated with increased risk of fatal outcomes in such patients and therefore T cell depletion has been used in

these disorders to prevent GVHD. TCRαβ+/CD19+ depleted allogeneic HSCT will likely provide a safer alternative to CD34+ selection in these high-risk patients even in the setting of fully matched URDs.

III. PREVIOUS WORK DONE IN THIS AREA

Multiple methods of ex-vivo and in-vivo T-cell depletion have been reported in the HLA-haploidentical HSCT setting.[22,23] CD34+ stem cells can be positively selected and administered as a graft product, or negative selection can be performed to remove specific components such as T cells from the graft product. CD34+ positive selection, or combined CD3+ and CD19+ negative selection, results in the loss of cells that are important for preventing infections and relapse.[9,10,24-26] In order to reduce the risk of infections and relapse, several centers have more recently implemented a TCRαβ+ T-cell/CD19+ B-cell depletion approach.[11] Selective TCRαβ+/CD19+ depletion aims to remove TCRαβ+ T-cells from the graft which have alloreactive potential and can cause GVHD, as well as antigen-presenting B cells which also contribute to GVHD.[27-30] However, TCRγδ+ T-cells purposefully remain in the graft to facilitate engraftment, reduce the risk of infections, and reduce the risk of leukemia relapse without causing GVHD as TCRγδ+ T-cells do not have alloreactive capacity.[18,31-34] Donor NK cells also remain in the graft and offer activity during the early weeks following HSCT until mature donor graft-derived NK cell reconstitution occurs.[16,35] The large number of stem cells typically infused with this type of product can also allow early robust neutrophil engraftment which may decrease early bacterial infections.

Promising clinical results have been recently reported in children and young adults treated with TCRαβ+/CD19+ depleted HSCT (Table 1). Bertaina et al evaluated 23 children with non-malignant hematological disorders who underwent haploidentical transplantation using TCRαβ+/CD19+ depletion.[36] No acute GVHD prophylaxis was administered. All but 4 of the patients engrafted. Three patients developed grade I-II acute GVHD of the skin, and there was no occurrence of visceral acute or chronic GVHD. The 2-year probability of disease-free survival was 91.1%. Other pediatric experiences have been published since, and are also summarized in Table 1.[19-21,36,37] All patients received less than 2x10^5 TCRαβ+ T cells per kg and the median CD34+ cell doses were 9x10^6/kg or greater. Rates of Grades 3-4 acute GVHD were low. Several studies did not use additional GVHD prophylaxis. Overall survival at various timepoints ranged from 50-97%.

Table 1. PREVIOUS WORK DONE IN THE AREA

Study	Bertania et al[36]	Balashov[37]	Lang[19]	Maschan[20]	Locatelli[21]
Year	2014	2015	2015	2016	2017
PMID	24869942	26187864	26039210	26808573	28588018
No of pts	23	37	41	33	80
Adult /pediatric	Pediatric	Pediatric	Pediatric	Pediatric	Pediatric
Disease Indication	Non-malignant disorders	Primary Immune Deficiencies	ALL (20) AML(9) MDS/JMML (3) Solid tumors (4) Nonmalignant (5)	AML	ALL, AML
Donor	Haplo	MUD (n=27) Haplo (n=10)	⩾2 HLA loci-mismatched parents	MUD MMRD Haplo	Haplo
GvHD prophylaxis	None	Tac/MTX (n=34) Tac/MMF (N=2) CSA/MTX (n=1)	MMF until day +30	None	None
CD34+ Dose	15.8x10^6/kg (10.2-40.0)	11.7x10^6/kg (5.9-21.3)	14.9x10^6/kg	9x10^6/kg (3.8–21)	13.9x10^6/kg (6-40.44)
Alpha/beta Dose	4x10^4/kg (1-9.5)	10.6x10^3/kg (0.8-368)	16.9x10^3/kg	20x10^3/kg (2.9–126)	0.047x10^6/kg (0.002-0.099)
Gamma/delta Dose	9.4x10^6/kg (1.6-95.4)		11.0x10^6/kg	9x10^6/kg (1–30)	8.1x10^6/kg (0.86-56.7)
Neutrophil Engraftment	Day+13 (10-20)	Day +16 (11-28)	Day +10 (7-21)	Day +16	Day +13 (9-19)
GVHD	Grade 1–2: 3 pts No chronic	Grade 2: 7 pts Grade 4: 1 pt Chronic: 1 pt	Grade 2: 4 pts Grade 3-4: 6 pts Chronic: 9 pts	Grade 2-3:14 pts Chronic: 10 pts	Grade 1-2: 24 pts Chronic: 4 pts
Graft Failure	2 Primary 2 Secondary	2 Primary 8 Secondary	5		2
Malignancy Relapse			17 pts	10 pts	19 pts
Infection	Viral reactivations: 9	CMV: 16pts, 5 with disease	Not Reported	CMV Reactivation: 52% EBV Reactivation: 50%	Not reported
Survival	2yr OS 91% 2yr DFS 91%	2yr OS 97% 2yr EFS 68%	If in CR1-3: 100% If in active disease: 0%	2yr OS 68% 2yr EFS 60%	OS 72% 5 years

RATIONALE

Based on these results we propose the use of αβ T-cell/CD19+ B-cell depleted stem cells in mismatched related and unrelated donor HSCT for malignant and nonmalignant disorders in pediatric and adolescent/young adult patients. Mismatched related donors provide an allogeneic HSCT option for essentially all patients in need of HSCT. This approach reduces the risk of acute GVHD and reduces the need for GVHD prophylaxis, either of which is poorly tolerated by certain patient groups. Additionally, transplantation can be performed without delay which can be critical in patients with high risk malignant disease or very severe non-malignant disorders requiring urgent treatment.

IV. RESEARCH PLAN

1a. How many subjects will be studied?

We will enroll up to 80 patients.

1b. How will subjects be selected for this study?

Patients attending the CCHMC bone marrow transplant (BMT) clinic who meet the eligibility criteria will be offered the opportunity to participate in this research study. The study is open to all participants regardless of gender or ethnicity.

2a. Inclusion Criteria

Any patient requiring an allogeneic HSCT who lacks an HLA-genotypically matched related donor. Genotypically matched related donors are allowed when there is a clinical desire to avoid the use of GVHD prophylaxis medications.

2b. Exclusion Criteria

Prior allogeneic transplant with active acute or chronic GVHD, or life-threatening infection. Patients with a prior history of allogenic transplant without active GVHD or life-threatening infection can be considered.

2c. Patients Requiring Second Allogeneic HCT or Additional Aliquots of Stored Hematopoietic Stem Cell Product

Patients may receive additional TCRa/b CD19 depleted product(s) if clinically indicated.

3. Randomization
Not applicable.

4. Procedures

Pre-Infusion Patient Evaluation:
Patients enrolled on this protocol will undergo evaluation for disease status and organ function per institutional HSCT guidelines prior to starting conditioning regimen.

Donor Selection:
Transplant donors will be selected based on institutional guidelines/SOPs.

Treatment Plan:
[redacted]

Informed Consent:
Informed consent will be obtained for each study participant (stem cell transplant recipient) and/or parent or legal guardian as appropriate. The study will be explained to the study subject in lay terms, in their native language. The informed consent process will require written consent by the patient (if ≥18 yrs of age) or parent/legal guardian.

The investigator will be available to answer any questions that the patient or caregiver may have regarding procedures, risks and alternatives. The signed consent form will be maintained in the study subject's chart with a copy being placed in the patient's medical record.

Preparative therapy:
Chemotherapy +/- radiation therapy +/- serotherapy will be administered to the recipient prior to infusion of donor stem cells based on patient's underlying diagnosis and organ function according to institutional standard of care or per study requirements if participating in another study. This will include myeloablative, non-myeloablative, reduced intensity, or minimal intensity conditioning regimens on or off study. On a rare occasion no preparative conditioning will be administered at the discretion of patient's primary BMT attending/team.

Donor Consent and Mobilization:
The stem cell donor will consent to collection of peripheral blood stem cells following standard clinical institutional guidelines/SOPs as the mobilization and collection of peripheral blood stem cells does not constitute research. Prior to initiation of the stem cell collection process, donors will be thoroughly evaluated and determined to be an acceptable donor candidate and able to tolerate all of the procedures involved. This will be done on a clinical basis per current standard-of-care. G-CSF mobilization of the donor will also be performed per institutional guidelines and SOPs.

Collection Target Cell Dose:
We will aim to collect 10 x 10^6 or greater CD34+ stem cells per kg recipient body weight.

Stem Cell Product Processing – TCRαβ+/CD19+ Depletion with CliniMACS (Miltenyi Biotech):
The TCRαβ+ T cells and the CD19+ B cells will be removed from the allogeneic graft utilizing the CliniMACS® immunomagnetic selection device (Miltenyi Biotec). The detailed procedures are provided in the CliniMACS® User Manual that accompanies the CliniMACS® Instrument. The depletion process involves two phases; cell labeling (phase 1) and the automated immunomagnetic depletion process (phase 2). A brief summary of the procedure is provided below.

Phase 1 – Labeling:

[redacted]

Phase 2 – Cell Depletion:

[redacted]

Post depletion - Stem Cell Dosing:

[redacted]

[redacted]

[redacted]

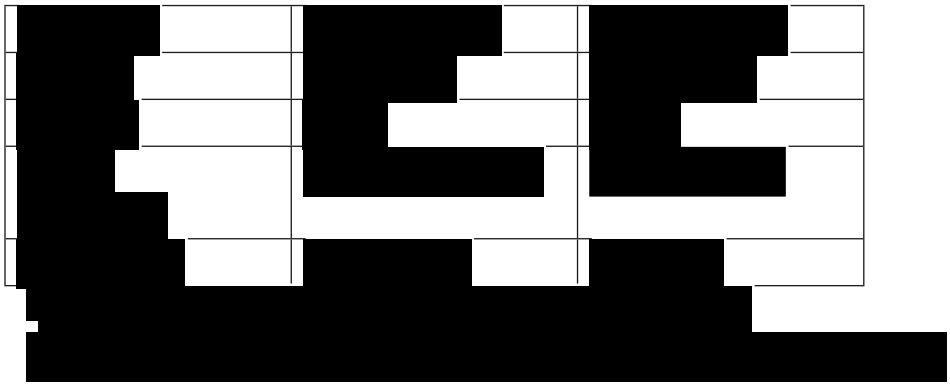
[redacted]

[redacted]

Stem Cell Processing:

[redacted]

Product Release Parameters:



Possible Interventions Based on Viable TCR alpha/beta T Cell Dose and B Cell Dose:

[redacted]

Safety of Product:

[redacted]

Product testing:

[redacted]

Culture of the product:

[redacted]

Stem Cell Infusion:

[redacted].

Immune Suppression after stem cell infusion:

[redacted]

[redacted]

Supportive therapy:

- [redacted]

Required Observations:

[redacted]

Day of Graft Infusion: [redacted]

Post-HSCT: [redacted]

[redacted]

V. EVALUATION CRITERIA

Primary Endpoint:

The primary endpoint of the study is safety of infusion of TCRαβ+/CD19+ depleted donor grafts.

We will determine the frequency and characteristics of potential infusion-related toxicity. Experience at our center using CD34+ selected stem cells has shown infusion related toxicity occurs at an incidence of <5%. We previously observed zero infusion reactions in 22 patients who received a CD34+ selected boost product as part of a CCHMC study (IRB# 2010-2344), and 2 infusion reactions among 55 infusions which were performed as part of clinical care including 1 occurrence of hypertension, and 1 occurrence of urticaria that resolved with anti-histamine administration. While there is little expectation of infusion-related toxicity, the incidence of infusion-related reactions will be recorded and evaluated.

Secondary Endpoints:

The secondary endpoints of the study are to assess engraftment and acute GVHD incidence.

Engraftment:
Engraftment and graft function are monitored on a clinical basis. Neutrophil recovery will be defined as the first of 3 consecutive days that the absolute neutrophil count is 500 cells/mcL. Platelet recovery will be defined as the first of 3 consecutive days that the platelet count is greater than 20 without any platelet cell transfusions in the previous 7 days. Initial donor-derived hematopoietic cell recovery will be defined as having 95% or greater donor-derived cells. Initial mixed donor and recipient-derived engraftment will be defined as having less

than 95% donor-derived cells but more than 5% donor-derived cells on a first and second confirmatory engraftment study (if the second study shows ≥95% donor cells, the patient will be classified as having donor-derived hematopoietic cell recovery). Recipient-derived hematopoietic cell recovery will be defined as having 5% or less donor-derived cells. Any patient who fails to achieve neutrophil recovery within 42 days of allogeneic HSCT, or has 5% or less donor-derived cells will be considered as having primary graft failure. We will report the cumulative incidence of achieving initial donor-derived hematopoietic cell recovery. Complete blood counts and whole blood donor chimerism studies are frequently performed on a clinical basis following allogeneic HSCT. Stable hematopoietic cell recovery at 100 Days (+/- 2 weeks) will be defined as having hemoglobin, neutrophil, and platelet counts of at least 80% of the normal range in the absence of transfusions or clinical diagnoses which may affect the counts such as hemolytic anemia, auto-immune thrombocytopenia, iron deficiency, infection or medication induced bone marrow suppression, or other clinically relevant diagnosis. Patients with these or other medical diagnoses may still be considered to have count recovery. The percentage of donor cells will be recorded at Day +100 (preferably +/- 2 weeks) and Day +100 Donor-derived hematopoietic cell recovery will be defined as having 95% or greater donor-derived cells. Day +100 mixed donor and recipient-derived hematopoietic cell recovery will be defined as having less than 95% donor-derived cells but more than 5% donor-derived cells. Recipient-derived Day +100 hematopoietic cell recovery will be defined as having 5% or less donor-derived cells. These patients will be considered as having secondary graft failure. We will report rates of successful donor-derived hematopoietic cell recovery at Day +100. Exceptions may be made for patients with Severe Combined Immune Deficiency who may reconstitute with donor-derived T cells but have autologous recovery of myeloid and other cells. For such cases we will examine results of sorted T cell chimerism studies. We will also record the incidence of any additional cell product administrations or second allogeneic HSCT. Any patient who receives a second allogeneic HSCT will be considered as having graft failure.

We will report the Day +100 event-free survival of patients where an event is defined as death, primary graft failure, autologous recovery (<5% donor-derived cells in whole blood or in the cellular compartment of interest, i.e. T cells in patients with SCID), secondary graft failure, receipt of an additional hematopoietic cell

product other than for control of infection (Donor lymphocyte infusion or Virus-Specific T cell infusion), and second allogeneic HSCT.

Acute GVHD:
Clinical assessments for acute GVHD are frequently performed using Modified Glucksberg Criteria as in Appendix 1. We will collect clinical information regarding incidence and severity of acute GVHD. We will report the Day +100 cumulative incidences of acute GVHD grades II-IV and III-IV.

Chronic GVHD:
Clinical assessments for chronic GVHD are also frequently performed using NIH Consensus criteria. We will report the 1 year cumulative incidences of chronic GVHD.

GVHD-Free Survival:
Based on the presence or not of acute or chronic GVHD at 1 year, we will report GVHD-free survival at 1 year.

VI. OFF STUDY CRITERIA/CRITERIA FOR WITHDRAWING SUBJECTS

Subjects (or parents or legal guardians) have the right to withdraw from the study at any time and for any reason without prejudice to his or her future medical care by the physician or the institution.

Any subject who withdraws consent to participate in the study will be removed from further treatment and/or study observation immediately upon the date of request. The subject will be followed for safety purposes for at least 1 week after infusion of the stem cell product.

<u>Criteria for Removal from Protocol Therapy:</u>

- Patient withdrawal from protocol therapy

<u>Off Study Criteria:</u>

a) Death
b) Receipt of a second stem cell product
c) Lost to follow-up

VII. STATISTICAL CONSIDERATIONS

1. Sample Size and Study Duration:

We chose a sample size of 80 patients based on estimates of patient need for access to T cell depleted grafts over 3-5 years at our Institution. All patients will be followed for primary and secondary endpoints for at least 100 Days following allogeneic HSCT, and 1 year data will be collected regarding survival and presence or absence of acute or chronic GVHD.

2. Statistical Considerations for Data Analysis:

Data will be presented with descriptive statistics. Cumulative incidence curves will be created to analyze engraftment and acute GVHD. Death will be treated as a competing event. Probability of overall survival and event-free survival will be estimated with Kaplan-Meier curves.

3. Stopping Rules:

A predictive probability design (Lee, Lin. Clinical Trials. 2008) will be used to control for safety with respect to TRM. The unacceptable rate of TRM is 30% or more. This will be tested against a one-sided alternative. The trial will initialize by enrolling 10 patients. If 3 or more out of 10 have a TRM the trial will stop. Otherwise the trial will continue according to the stopping rules plan (Appendix 2) up to a maximum of 73 patients. If the number of patients with a TRM within 100 days following allogeneic HCT is ever greater than or equal to the number listed in the corresponding patients enrolled row the trial will stop and the drug will be declared not safe. This design yields a type I error rate 0.05 and power greater than 80% when the true rate of TRM is 15%. Note the stopping rule must be made based on the chronological order of the patients enrolled.

We plan to meet with medical monitor approximately every quarter to review these safety data and stopping rules. The medical monitor will review the TRM data in the context of HLA mismatch; and as usual, he/she will have the final authority regarding continuation of the study in the event of excessive TRM.

VIII. REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

The protocol and informed consent document for this study must be approved in writing by the Institutional Review Board (IRB) prior to any patient being registered on the study.

Changes to the protocol must also be approved by the IRB. Records of the Institutional Review Board review and approval of all documents pertaining to this study must be kept on file by the investigator and are subject to FDA inspection at any time during the study. Periodic status reports will be submitted to the Institutional Review Board at least yearly, as well as notification of completion of the study and a final report within 6-12 months of study completion or termination. Informed consent will be obtained prior to the treatment of participants.

1. Data Safety Monitoring Plan:

The study will be monitored closely by a medical monitor. This will be a qualified physician not associated with this particular protocol. The monitor will have expertise in basic hematology and conduction of clinical trials, and will have prior regulatory experience for similar studies. The monitor will work closely with the PI to monitor the participants on study. He/she will meet with the PI and review study data/progress approximately every 3 months; more often at the discretion of the PI and/or medical monitor.

The medical monitor will review the TRM data in the context of HLA mismatch; and as usual, he/she will have the final authority regarding continuation of the study in the event of excessive TRM. The medical monitor is required to review all unanticipated problems involving risk to subjects or others, unanticipated serious adverse events and all subject deaths associated with the protocol and provide an unbiased written report of the review.

At a minimum, the medical monitor should comment on the relationship of the event or problem, to participation in the study. Based on the review of these events, the monitor should make a recommendation regarding study continuation. Although the expected rate of serious toxicities will be low, any unanticipated attributable serious adverse event will be discussed with the medical monitor. If there are any attributable Serious Adverse Events, the study will stop accrual

pending review by the medical monitor. The study will resume patient accrual only if written approval is given by the medical monitor.

All decisions regarding study continuation, modification, or termination will be reported immediately or annually, as appropriate, to the IRB and other appropriate agencies. Reports for events determined by either the investigator or medical monitor to be possible or definitely related to study participation and reports of events resulting in death should be forwarded in compliance with current IRB policy and applicable federal regulations.

All reports from Medical monitor will be forwarded to Cincinnati Children's Hospital Medical Center IRB and other regulatory agencies as appropriate.

2. Blood Specimens:
There are no research blood specimens for this study.

3. Other Previously Approved Research Studies in Which the Projected Patient Population May Also Be Involved:
Patients may be additionally involved in other studies surrounding the care of patients undergoing allogeneic HCT.

4. Data Collection and Monitoring:
Data for the study will be entered into a secure, password protected database by the study staff. Monitoring and auditing procedures will be followed to ensure that the study is conducted, documented, and reported in accordance with the IRB approved protocol, all applicable federal regulations and guidelines, and applicable regulatory requirements of Cincinnati Children’s Hospital Medical Center.

Verification of eligibility will be performed and appropriate documentation of informed consent will be documented for all subjects enrolled into the study. The timeliness of Serious Adverse Event reporting will be monitored to ensure regulatory compliance. All case report forms (CRF) for the first subject enrolled into the study will be monitored for completeness and quality by comparing data in the case report forms to data in the source documents. Thereafter, a minimum of 10% of enrolled subjects’ CRFs will be monitored for completeness and quality by comparing data in the case report forms to data in the source documents.

5. Facilities Utilized in the Study:
Study participants will be cared for by the Division of Bone Marrow Transplantation and Immune Deficiency physicians on the Bone Marrow Transplant inpatient and outpatients units at Cincinnati Children's Hospital Medical Center.

IX. POTENTIAL BENEFITS

Patients may benefit from this study from gaining access to allogeneic HCT with a reduced risk of acute GVHD.

X. POTENTIAL RISKS, DISCOMFORTS, INCONVENIENCES AND PRECAUTIONS

1. Potential risks, discomforts, inconveniences specific to the study agent administration and research evaluations include:
There is a risk of infusion reactions that we estimate to be less than 5% based on previous experience using CD34+ selected stem cell grafts. Every effort will be made to minimize any discomfort associated with TCRαβ+/CD19+ depleted stem cell infusion.

There is also risk of loss of confidentiality. We will minimize this risk by keeping all study data stored on password protected computers. Any paper records will be kept in secure office locations. Access to the files will be limited to the Principal Investigator and designees.

2. Carcinogenesis, Mutagenesis, and Impairment of Fertility:
Selection of the hematopoietic stem cell product should not affect these complications. Any conditioning chemotherapy prescribed on a clinical basis (not part of the study) may affect risk of these complications and is explained during the clinical informed consent procedure for clinical treatment with allogeneic HCT.

3. Pregnancy/Lactation:
Selection of the hematopoietic stem cell product should not affect risk to pregnancy/lactation. However due to the high risk nature of allogeneic HCT, patients should not be pregnant or lactating.

4. Pediatric Use:
This study will enroll pediatric and adolescent and young adult patients.

5. Adverse reactions:
There is a risk of infusion reactions that we estimate to be less than 5% based on previous experience using CD34+ selected stem cell grafts. To minimize this risk, pre-medication with anti-histamines and/or steroids will be used before infusion as per standard clinical guidelines. Every effort will be made to minimize any discomfort associated with TCRαβ+/CD19+ depleted stem cell grafts.

6. Precautions that will be taken to monitor and avoid the above mentioned risks, discomforts, and inconveniences:
Patients will be clinically monitored during the infusion of TCRαβ+/CD19+ depleted stem cell grafts and any complications will be clinically treated.
All study data will be stored on password protected computers. Any paper records will be kept in secure office locations.

7. Adverse Events:
Unanticipated severe adverse event/s must be reported to the principal investigator who is responsible for the reporting to the Cincinnati Children's Hospital Medical Center IRB, FDA, and the medical monitor as applicable.

All Adverse Event reporting is to comply with the current CCHMC IRB policy and applicable federal regulations. If any serious adverse events occur, current guidelines will be followed for expedited reporting to the Sponsor, IRB, FDA and/or the medical monitor.

The investigator should notify the IRB of serious adverse events occurring at the site, in accordance with local procedures.

Definitions:

Adverse Event
An adverse event is any new, undesirable medical occurrence or change of an existing condition in a subject that occurs during treatment (which may include a specified post treatment period), whether or not considered to be product related. Abnormal laboratory findings considered by the investigator to be clinically significant, e.g. those that are unusual or unusually severe for the population being studied, should be recorded as adverse events.

Serious Adverse Event

A serious adverse event is defined by regulatory/clinical criteria. It is one that suggests a significant hazard or side effect, regardless of the investigator's or sponsor's opinion on the relationship to study material. This includes, but may not be limited to, any event that (at any dose):

- is fatal;
- is life threatening (places the subject at immediate risk of death);
- requires intensive care;
- is persistent and results in significant disability/incapacity;
- Hospitalization
- Disability or Permanent Damage
- Congential Anomaly/Birth Defect
- Require Intervention to Prevent Permanent Impairment or Damage (this one may only apply to investigational devices)
- Other Serious (Important Medical Events)
- For an event that may not be immediately life threatening or result in death or hospitalization, but may jeopardize the patient or require intervention to prevent one of the outcomes listed above, or result in urgent investigation, may be considered serious.

Important medical events that may not be immediately life threatening or result in death or hospitalization, but may jeopardize the patient or require intervention to prevent one of the outcomes listed above, or result in urgent investigation, may be considered serious.

8. Adverse Event Reporting:

Safety and tolerability will be assessed according to the Common Terminology Criteria for Adverse Events v5.0 (CTCAE published November 27, 2017). The principal investigator(s) or designee will review adverse events closely and assess the event's relationship to study procedures to determine whether the event is unrelated or, unlikely, possibly, probably or definitely related to the study procedures, especially in context of the underlying disease and baseline lab values for the patient.

Allogeneic HSCT is known to be a high-risk procedure with many complications and a gross mortality risk of approximately 70%. Only adverse events of grade 4 or

higher will be recorded on case report forms. Because the primary aim of this study is to assess safety of the infusion of TCRαβ+/CD19+ depleted HSCT, and infusion reactions or other adverse events are expected to occur within 48 hours of the initial product infusion, we will only monitor and record AEs for the first week of the study. Any "attributable" events noted after this first week will be recorded and reported according to applicable institutional and federal regulations. Patients will be followed through Day +100 for endpoints including engraftment, acute GVHD, and event-free survival.

Expected events are those that have been previously identified as resulting from administration of the protocol therapy, those that can be attributed to the underlying condition, and/or those associated with transplant. An adverse event is considered unexpected, for reporting purposes only, when either the type of event or the severity of the event is not listed in the protocol and/or investigational brochure.

Please note that for the purpose of this study we will exclude the report of hematologic toxicities, as these subjects, by virtue of their underlying disease and/or condition and the transplant regimen, will develop hematologic abnormalities that otherwise would qualify as toxicity. Hematologic toxicities which are attributable to the underlying hematological disease and/or HCT preparative regimen will not be considered adverse events. In the event that any of these known complications of transplant or hematologic toxicities are thought to be at least possibly attributable to the study, the event will be recorded and subsequently reported to the IRB and FDA according to current reporting requirements.

Additionally for this study, events as previously identified as resulting from administration of a stem cell transplant will also be considered expected. The transplant regimen is well known to commonly affect systems such as the hematologic, immunologic, and gastrointestinal systems. The following are known complications of transplant: feeling tired, anemia, disseminated intravascular coagulation, febrile neutropenia, hemolysis, thrombotic thrombocytopenia purpura, adrenal insufficiency, colitis, constipation, diarrhea, enterocolitis, gastrointestinal pain, malabsorption, mucositis, nausea, pancreatitis, vomiting, dyspepsia, hepatitis, jaundice, hepatic veno-occlusive disease, thrombotic microangiopathy, fever, pain (including but not limited to headache, muscle

cramps, abdominal pain, throat pain etc), cholecystitis, allergic reaction, serum sickness, entercolitis, infections, viral reactivations, upper respiratory infection, urinary tract infection, leukocytopenia, lymphocytopenia, neutropenia, thrombocytopenia, bleeding complications such as epistaxis, hematemesis, petechiae, subconjunctival hemorrhage, weight gain/loss, fluid retention, acidosis, alkalosis, anorexia, dehydration, glucose intolerance, electrolyte disturbances, iron overload, generalized muscle weakness, reversible posterior leukoencephalopathy syndrome, bladder spasm, cystitis, noninfective, hematuria, urinary frequency, urinary urgency, irregular menstruation, pruritis, hypertension, depression, anxiety, purpura, and petechiae. It is notable that adverse events following allogeneic hematopoietic cell transplantation can be severe and life-threatening. Acute graft versus host disease, infections, mixed chimerism, cytopenias, and graft failure are all known complications of allogeneic HCT, and can result in death. Organ failure my also occur due to toxicities of chemotherapy, infections, thrombotic microangiopathy, or other reasons. For the purposes of this study, the listed events that are </= grade 3 will not be considered adverse events. All listed events that are grade 4 or 5 will be recorded on the AE case report forms (CRF) and reported according to current IRB guidelines. In the event that any of these known complications are thought to be at least possibly attributable to the study procedure, and not to the allogeneic hematopoietic cell transplantation and expected complications, the event will be recorded and subsequently reported to the IRB and FDA according to current reporting requirements. Acute GVHD, mixed chimerism, and graft failure are not considered AEs during the study (they are captured as endpoints).

Medical conditions/diseases present before the infusion of TCRαβ+/CD19+ depleted HSCT are only considered adverse events if they worsen after the infusion of TCRαβ+/CD19+ depleted HSCT. Abnormal laboratory values or test results constitute adverse events only if they induce clinical signs or symptoms, are considered clinically significant, or require therapy.

Adverse Events will be reported according to current CCHMC IRB policy and applicable federal regulations. If any serious adverse events occur, current guidelines will be followed for expedited reporting to the IRB, medical monitor, and sponsor.

- If an event meets all of the below criteria, an IND Safety Report will be generated and submitted to the FDA by the sponsor no later than 15 calendar days after the sponsor receives the safety information and determines that the information qualifies for reporting:Suspected Adverse reaction
- Serious
- Unexpected

9. Risk assessment recommendation:

☐ Minimal risk but with direct benefit to participants
☐ Minimal risk but without direct benefit to participants
☒ More than minimal risk, but potential direct benefit to participants
☐ More than minimal risk but without direct benefit to participant

XI. CONFIDENTIALITY

All records and documents pertaining to the study will be maintained by the Investigator and designee(s), and will be available for inspection by the Cincinnati Children's Hospital Institutional Review Board, the sponsor and the Medical monitor. All documents will be kept in a locked cabinet or on password protected computers in the office of the Clinical Management Research Support Core (CMRSC). Access to the files will be limited to the Principal Investigator and designees. All information provided to the investigator dealing with the study and information obtained during the course of the study will be regarded as confidential.

XII. PERIOD OF TIME ESTIMATED TO COMPLETE PROJECT AS DESCRIBED

The period of time to complete enrollment for this study is estimated to be approximately 5 years. Total time for completion of follow up is estimated to be 6 years.

XIII. FUNDING

The institution to which funding will be made: CCHMC

XIV. PAYMENT FOR STUDIES

1. Third party payers

All costs associated with the routine clinical care of patients will be the responsibility of the patient or their insurance carrier.

2. Reimbursement to participants

The patients will receive no financial compensation for participation in this research protocol. All study visits will be scheduled at the same time clinical visits are planned.

The FDA authorized cost recovery for this study. The FDA determined that the cost of Alpha Beta CD19 Depleted Stem Cell graft using Miltenyi Biotech CliniMACS Immunomagnetic Selection Device may be billed to patients and/or their insurance company.

XV. METHOD TO BE USED IN PROCURING CONSENT OF SUBJECTS

All prospective patients will have the study explained by a member of the research team. All the potential hazards and possible adverse reactions will be explained to patient/parent.

Prior to the initiation of the study, acknowledgement of the receipt of this information and the subject's freely tendered offer to participate will be obtained in writing from each subject in the study. Assent for subjects 7-10 years old will not be obtained due to the complex nature of the therapy and the limited capability of the minor to reasonably consider all the implications associated with this treatment that may be of direct benefit to the subject.

This protocol, informed consent, assent form, and any amendments to the protocol will be reviewed and approved by the CHMCC IRB prior to initiation. The study will not be initiated without the approval of the IRB.
At reaching the age of majority (typically 18 years of age), an attempt will be made to reconsent the research participant at their next visit to the CCHMC BMT clinic.

If it will be more than 12 months from the time of their 18th birthday to their next planned visit, contact via phone and via mail will be made for their consideration

to continue participation. The call and letter will explain that an attempt will be made to reconsent the research participant at their next visit. In addition, study staff contact information will be provided in case they have questions or wish to inform us of their desire to withdraw from participation prior to their next planned visit.

XVI. REFERENCES

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APPENDIX 1. ACUTE GVHD GRADING SCALE – MODIFIED GLUCKSBERG INDEX

Grading of acute GVHD will be based on the following 2 tables and recorded as I, II, III, or IV as defined in table 2.

Table 1. Staging of Organ Systems in aGVHD

Stage	Skin	Liver (Bilirubin)	Intestinal tract (Diarrhea)
0	No rash	<35 *µmol/l* or <*2.0* mg/dl	None or <500 ml/day or 280ml/m^2/day
1	Maculopapular rash, <25% of body surface	35-52 *µmol/l* or *2.0-3.0* mg/dl	500-1000 mL/day or 280-555ml/m^2/day
2	Maculopapular rash, 25-50% of body surface	53-103 *µmol/l or 3.1*-6.0 mg/dl	1000-1500 ml/day or 556-833 ml/m^2/day
3	Generalized erythroderma	104-256 *µmol/l* or *6.1-15.0* mg/dl	>1500 ml/day or >833ml/m^2/day
4	Generalized erythroderma with bullae formation and desquamation	>256 *µmol/l or >15.0* mg/dl	Severe abdominal pain with or without ileus

- Use burns chart or rule of nines:
  - Arm (all over) 9% Front 18% Head 9% Palm 1% Leg (all over) 18% Back 18% Genitals 1%
- Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented.
- If urine contamination suspected do not count
- General total volume of diarrhea applies for adults. Downgrade one stage if an additional cause of diarrhea has been documented.
- Histological evidence of aGVHD in the stomach or duodenum.

Table 2. Glucksberg grading of aGVHD

	Skin	Liver	Intestinal Tract
Grade 0	Stage 0	Stage 0	Stage 0
Grade I	Stage 1-2	None	None
Grade II	Stage 3	Stage 1	Stage 1
Grade III		Stage 2-3	Stage 2-4
Grade IV	Stage 4	Stage 4	-

APPENDIX 2: STOPPING RULES PLAN

Patients Enrolled	Stop if # with TRM within 100 days following HCT is greater than or equal to
10	3
11	3
12	3
13	4
14	4
15	4
16	5
17	5
18	5
19	5
20	6
21	6
22	6
23	6
24	7
25	7
26	7
27	7
28	7
29	8
30	8
31	8
32	8
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38	10
39	10
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41	10
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43	11
44	11
45	11
46	11

47	12
48	12
49	12
50	12
51	12
52	12
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54	13
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59	14
60	14
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62	14
63	14
64	14
65	15
66	15
67	15
68	15
69	15
70	15
71	15
72	15
73	15