Official Study Title: TCRαβ-DEPLETED PROGENITOR CELL GRAFT WITH ADDITIONAL MEMORY T-CELL DLI, PLUS SELECTED USE OF BLINATUMOMAB, IN NAIVE T-CELL DEPLETED HAPLOIDENTICAL DONOR HEMATOPOIETC CELL TRANSPLANTATION FOR HEMATOLOGIC MALIGNANCIES

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TCRαβ-DEPLETED PROGENITOR CELL GRAFT WITH ADDITIONAL MEMORY T-CELL DLI, PLUS SELECTED USE OF BLINATUMOMAB, IN NAÏVE T-CELL DEPLETED HAPLOIDENTICAL DONOR HEMATOPOIEITC CELL TRANSPLANTATION FOR HEMATOLOGIC MALIGNANCIES

IDE #16971

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TABLE OF CONTENTS

STUDY SUMMARY

Protocol Title

TCRαβ-depleted progenitor cell graft with additional memory T-cell DLI, plus selected use of blinatumomab, in naïve T-cell depleted haploidentical donor hematopoietic cell transplantation for hematologic malignancies

Principal Investigator:

Brandon M. Triplett, MD

Brief Overview

Study Population:

Patients less than or equal to 21 years old with high-risk hematologic malignancies who would likely benefit from allogeneic hematopoietic cell transplantation (HCT). Patients with a suitable HLA matched sibling or unrelated donor identified will be eligible for participation ONLY if the donor is not available in the necessary time.

Additional eligibility criteria are specified to assure sufficient multi-organ system function.

Intervention, Brief Outline, and Objectives of Treatment Plan:

In this study, participants with high-risk hematologic malignancies undergoing hematopoietic cell transplantation (HCT), who lack an available suitable human leukocyte antigen (HLA) matched related/sibling donor (MSD) or matched unrelated donor (MUD), will receive a TCRαβ-depleted haploidentical donor HCT with additional memory cell DLI. One course of blinatumomab will be empirically added for patients with CD19+ malignancy.

The assessments and follow-up evaluations noted in the protocol follow the St. Jude standard operating procedures (SOP) for all recipients of allogeneic HCT.

The primary objective of the study for the original cohort is to determine the minimum effective dose for prophylactic CD45RA-depleted DLI when given in the early post-engraftment period. In addition, we will assess efficacy of TCRαβ-depleted progenitor cellgraft with additional memory T cell DLI, plus selected use of blinatumomab, in haploidentical donor hematopoietic cell transplantation for hematologic malignancies as measured by 1 year EFS (events = relapse, death). For the Amendment 5 expansion (early DLI) cohort, the primary objective is to assess the feasibility and safety of early CD45RA-depleted DLI administration.

Secondary and Exploratory aims will assess overall survival, event-free survival, risk of relapse, graft versus host disease (GVHD), transplant related mortality (TRM), transplant related morbidity, and immune reconstitution.

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Criteria for Evaluation – Safety and Efficacy

Safety:

The primary measures of safety will be the rate of therapy related death and the rate of severe graft versus host disease.

Ongoing assessment of toxicity will be done using the NCI CTCAE version 5

Acute and chronic graft-vs.-host disease will be evaluated using established staging/grading criteria and expert consensus guidelines.

Efficacy:

The primary measure of efficacy for memory T-cell DLI will be achievement of a peripheral blood donor memory T-cell count of at least 300/μL at 4 weeks following the prophylactic DLI.

The primary measure of efficacy for the trial will be event-free survival at one year (events = relapse, death)

Neutrophil and platelet engraftment will be determined using the parameters put forth by the Center for International Blood and Marrow Transplant Research. Assessments will be made upon review of daily complete blood count and serial (typically weekly) chimerism studies.

Bone marrow studies for disease status evaluation will be performed at approximately 21 days, 100 days, and 1 year post-transplant. Testing will include evaluations for minimal residual disease.

Immune reconstitution will be determined by standard blood testing of lymphocyte subsets. Research tests required for participation will examine lymphocyte phenotype and function.

Statistical Considerations and Data Analysis

Study Design: Phase II

Randomization: No

Sample Size: Initial study: Up to 70; Early DLI cohort: Up to 30

Data Analyses:

Anticipated primary completion date: 12/31/2026

Anticipated study completion date: 12/31/2027

Time frame for primary outcome measure: 1 year post-transplant

Data Management including statistical evaluations

Protocol compliance, data collection including safety data, and reporting will be carried out by the Department of Bone Marrow Transplantation and Cellular Therapy Research Office.

Statistical considerations and ongoing analysis will be conducted by Dr. Subodh Selukar and designated associates within the St. Jude Department of Biostatistics.

Human Subjects:

The risks to participants are primarily related to the cellular infusions and the conditioning regimen. The proposed regimen and donor cells may induce serious and possibly fatal disorders such as GVHD, veno-occlusive disorder, and post-transplant lymphoproliferative disease. Because of the required conditioning, recipients are at high-risk for serious and possibly life-threatening infection, bleeding, and anemia. Adverse events will be treated, monitored, and reported appropriately.

Possible benefits of participation include obtaining and/or sustaining disease remission. In addition, there is the possibility of psychological benefit from knowing participation has helped researchers gain more understanding about the efficacy of haploidentical HCT.

Alternatives to participation are identified as conventional (standard) transplantation, chemotherapy without transplant, including palliative therapy, other research treatment if available, and/or supportive therapy alone.

The possible benefits, alternatives to participation, and side effects, including that there may be unknown side effects of treatment, are detailed in lay language within the respective informed consent document.

1.0 OBJECTIVES

1.1 Primary Objective

1.1.1 Determine the maximum effective dose for prophylactic CD45RA-depleted DLI when given in the early post-engraftment period.

1.1.2 Assess the efficacy of TCRαβ-depleted progenitor cell graft with additional memory T-cell DLI, plus selected use of blinatumomab, in haploidentical donor hematopoietic cell transplantation for hematologic malignancies as measured by 1 year EFS (events = relapse, death)

1.1.3 Assess the feasibility and safety of early CD45RA-depleted DLI administration in the Amendment 5 expansion (early DLI) cohort

1.2 Secondary Objectives

1.2.1 Assess the safety and feasibility of the addition of blinatumomab in the early post-engraftment period in patients with CD19+ malignancy.

1.2.2 Estimate the incidence of neutrophil and platelet engraftment, malignant relapse, event-free survival per disease subgroups (e.g. ALL vs AML), and overall survival at one-year post-transplantation.

1.2.3 Estimate incidence and severity of acute and chronic (GVHD).

1.2.4 Estimate the rate of transplant related mortality (TRM) in the first 100 days after transplantation.

1.2.5 To measure and describe the pharmacokinetics of rabbit ATG in HCT recipients on this study.

1.3 Exploratory Objectives

1.3.1 Record immune reconstitution parameters, including chimerism analysis, quantitative lymphocyte subsets, T cell receptor excision circle (TREC) analysis, V-beta spectratyping, and lymphocyte phenotype and function.

1.3.2 Describe the use of additional CD45RA-depleted DLI for recipients who have severe viral infections, disease recurrence or progression, or poor immune reconstitution. Assess and record efficacy of CD45RA-depleted DLI for these conditions, and all adverse events that are related to CD45RA-depleted DLI.

1.3.2.1 Assess the degree of T-cell expansion in the early post HCT period and at 4 weeks post-DLI in the early DLI cohort compared to the original cohort.

1.3.2.2 Assess the cumulative incidence of composite viremia (including CMV, EBV, and adenovirus) in the early DLI cohort compared to the original cohort; and assess the duration of CMV viremia in the Amendment 5 early DLI cohort compared to the original cohort.

2.0 BACKGROUND AND RATIONALE

2.1 Overview

Allogeneic HCT is a potential curative therapy for various pediatric hematologic malignancies, however approximately 25-60% of eligible pediatric recipients will not have a human leukocyte antigen (HLA)-matched related/sibling donor (MSD) or a HLA matched unrelated donor (MUD).[1-4] The lack of an adequate MUD for a substantial number of patients, and the lengthy duration of the donor search process in those with high risk of relapse, have prompted the search for alternative donors, including haploidentical family donors and unrelated umbilical cord blood (UCB) grafts. [3,4]

Nearly all patients have a readily available mismatched family member (haploidentical) donor, and haploHCT is an effective therapy for patients with hematologic malignancies.[5,6] This institution has a track record of success using haploidentical donors for patients with hematologic malignancies.[7] However, in our early studies, profound T-cell depletion of these mismatched grafts was utilized for avoidance of GVHD – thereby prolonging donor adaptive immune recovery and attenuating T-cell mediated GVL. Recently, we have found that selective depletion of CD45RA+ cells provides efficient depletion of B cells and acute GVHD inducing naïve T cells, while preserving hematopoietic progenitor cells and memory T cells. Specifically, the recent study HAPNK1 utilized selective T-cell depletion of the donor progenitor cell graft with CD45RA-depletion, which provided adoptive transfer of diverse donor memory T cell populations. Although treatment related death and overall outcomes have been better than previous studies, there have been significant toxicity burden with cytokine release syndrome and severe acute GVHD in a subset of patients. There is also evidence in the literature that TCRγδ T cells likewise may facilitate engraftment and have anti-leukemia effects (see section 2.3). The combination of reduced intensity conditioning regimen, administration of donor TCRγδ T cells, and precise dosing of donor memory T cells has the potential to provide low regimen-related toxicity, a low rate acute and chronic GVHD, enhanced donor immune recovery to prevent prolonged immunodeficiency, and a low rate of disease recurrence, which would likely maintain the excellent disease-free survival seen in HAPNK1 and lead to an enhanced quality of life.

Herein, we propose a single institution prospective phase II trial to determine the maximum effective dose for CD45RA-depleted DLI when given in the early post-engraftment period; and to estimate the efficacy of TCRαβ-depleted haploidentical donor progenitor cell graft and CD45RA-depleted DLI following reduced intensity conditioning regimen that avoids radiation in patients with high risk hematologic malignancies This study will evaluate the safety and efficacy of this HCT procedure, through the estimation of donor engraftment, survival, relapse, acute and chronic GVHD, chimerism, toxicity, and disease recurrence.

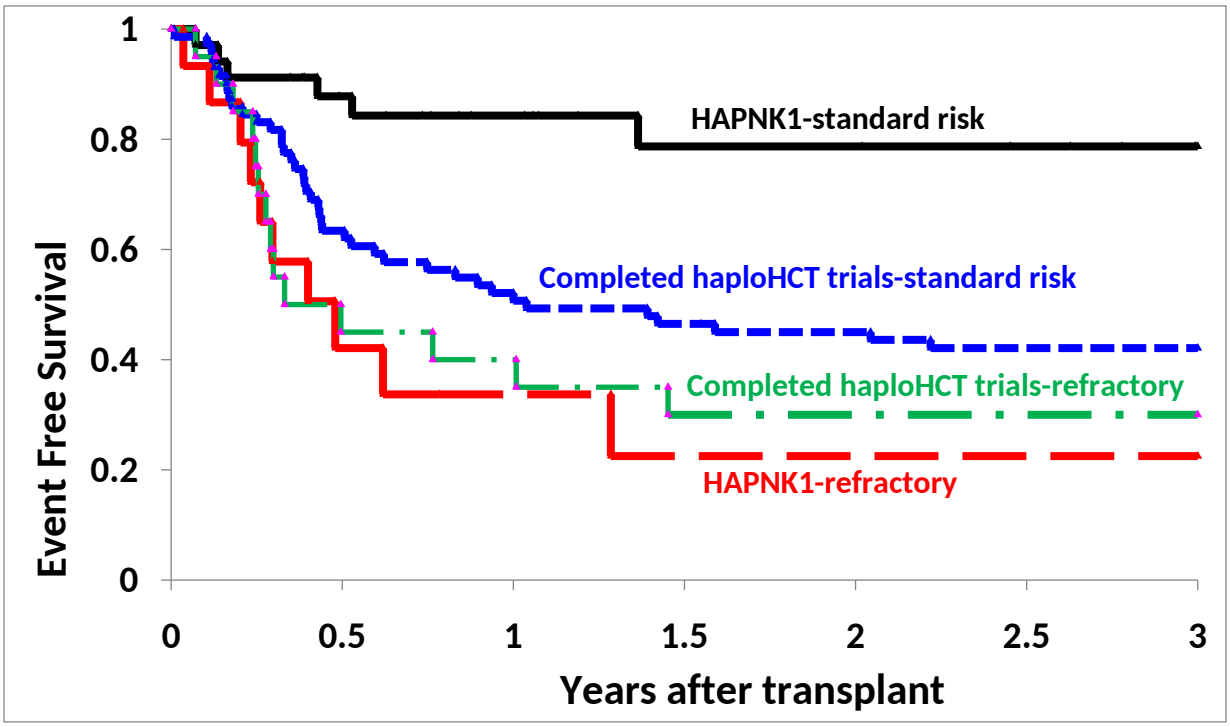
2.2 Haploidentical HCT

Haploidentical donors are viable alternative donors since family members are highly motivated and readily available for most patients. Along with other institutions, St. Jude has shown haploidentical HCT to be an effective therapy for patients with hematologic malignancies. Due to the high potential for GVHD with the degree of HLA mismatch seen in haploHCT, haploidentical grafts are often extensively T-cell depleted prior to infusion.

Early results with T-cell depleted haploHCT were disappointing due very high rates of graft failure and infections. Significant progress was initially achieved with the use of mega-dose of T-cell depleted hematopoietic progenitor cells (HPC) after high-intensity conditioning. Aversa and colleagues initially reported successful long-term engraftment with minimal GVHD in 43 patients with leukemia (including 15 children) using high doses of CD34+ enriched haploidentical cells, purified by positive (CD34+) and negative (CD3-lectin agglutination) selection. Utilizing haploidentical grafts and megadose cell therapy (>1 x 10^7 CD34+ cells/kg), Handgretinger and colleagues reported similar results in 23 pediatric patients. Prompt engraftment was associated with a low incidence of GVHD, even without the use of pharmacologic GVHD prophylaxis.

Building on the initial studies above, St. Jude has gained considerable expertise with haploidentical HCT in children. Over the past 16 years (2001-2016), 365 mismatched related (haploidentical) donor HCT have been performed at St. Jude. Initially, most haploHCT performed in this institution were for patients with relapsed and refractory hematologic malignancies, however as it became a more effective therapy, it has become a primary transplant option for patients in remission who lack a well matched donor.

The current haploHCT study HAPNK1 was activated in 2013. Preliminary results analyzed as of August 2017 (n=49) showed that early event free survival (EFS) for standard high risk (non-refractory) patients on HAPNK1 (black) was substantially improved compared to previously completed haploHCT trials (blue) at St. Jude, however the EFS for refractory patients on HAPNK1 (red) did not improve compared to previously completed haploHCT trials (green) (events = relapse, death).



Based on this experience, it is clear that refractory patients need a specialized protocol designed differnently and non-refractory (standard) HCT patients. Therefore, standard non-refractory high risk malignancies will be treated on this study, and refractory patients who are receiving their first allogeneic HCT will be transplanted on a specialized refractory (REF) protocol.

2.3 Rationale for Immunomagnetic T-Cell Depletion (TCD) of Hematopoietic Progenitor Cell Graft

As mentioned above, T-cell depleted haploidentical HCT were reasonably effective with the infusion of a large number of CD34+ cells (>10^7 CD34+ cells/kg). However, with little to no adoptive transfer of donor T-cell immunity, T-cell functional reconstitution was substantially delayed. Donor T cells in the haploidentical graft play a major role in mediating engraftment and GVL, but also GVHD - as few as 3 x 10^4 conventional T cells/kg can cause GVHD. Methods of TCD have been developed to generate a graft with very low T-cell content, such that the risk of GVHD is decreased. T cells can be removed from the graft by direct removal of T cells (negative selection) or selection of CD34+ progenitors (positive selection). The transplantation program at St. Jude has had success using either method of TCD in haploHCT.

Although positive selection allows for extensive TCD, the graft is devoid of other important cell populations such as NK cells, myeloid cells, dendritic cells, and monocytes. Several previous institutional haploHCT studies utilized CD3+ cell depletion by CliniMACS, which preserves these innate immune facilitating populations. This method substantially, but non-selectively, depletes all T-cell populations. Although GVHD inducing naïve T cells are adequately depleted, potentially beneficial T-cell populations that may not contribute substantially to GVHD such as gamma/delta (γ/δ) T cells and memory T cells are likewise depleted in similar scale.

A newer form of immunomagnetic TCD available is α/β T-cell depletion by CliniMACS. TCD of mobilized peripheral blood progenitor cell products has been shown to effectively and consistently deplete α/β T cells approximately 4 log (10,000 fold).[8,9] This level of α/β T-cell depletion is greater than the level of TCD with CD3+ cell depletion by CliniMACS. Importantly, this method has a CD34+ cell recovery comparable to the CD34+ enrichment method of TCD, and preserves a γ/δ T-cell dose >100 fold greater than the residual α/β T-cell dose. This is desirable because α/β T cells cause GVHD, and γ/δ T cells do not appear to cause GVHD.[10,11] In addition, there is evidence that γ/δ T cells have anti-leukemia effects, and may facilitate engraftment. Additionally, higher γ/δ T-cell recovery in the early post-HCT period has been associated with improved leukemia free survival.[12] Our experiences with TCRαβ-depletion performed by the St. Jude Human Applications Laboratory in two institutional protocols (REFNK1, REF2HCT) show similar results, with a greater than 90% recovery of CD34+ cells, CD56+ cells, and γ/δ T cells, and with a greater than 4 log depletion of α/β T cells.

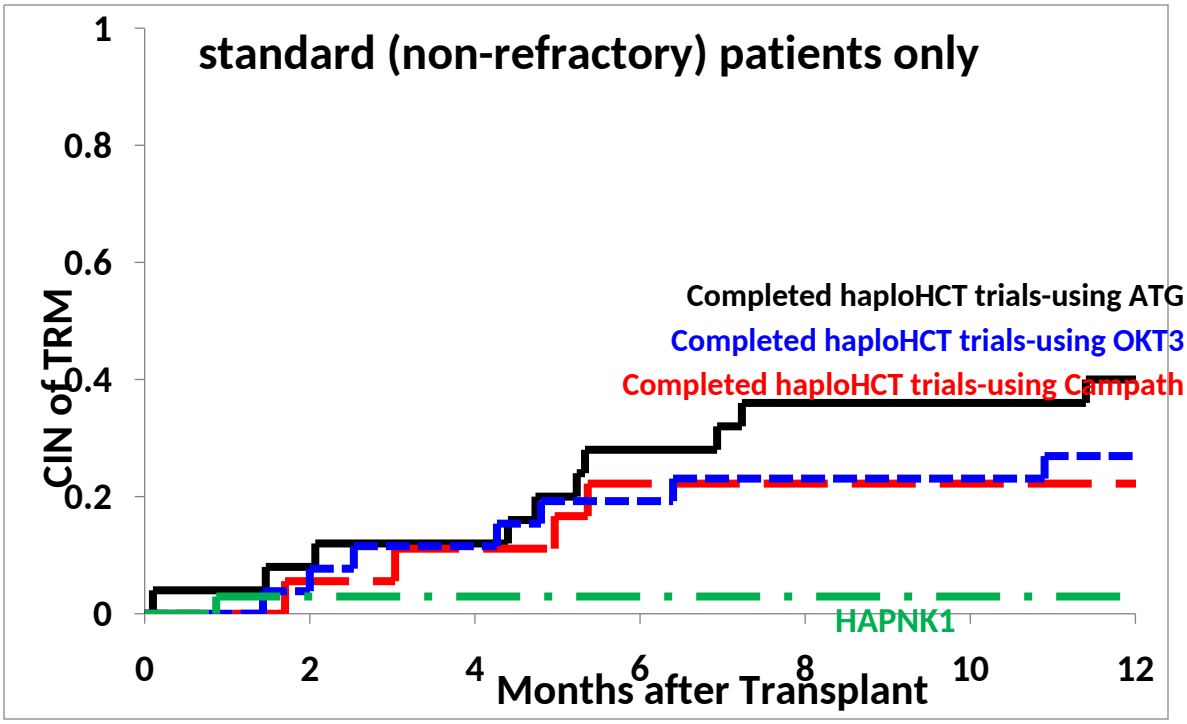
There is extensive publication record for use of TCRαβ-depleted haploidentical donor progenitor cell grafts in HCT for patients with malignant and nonmalignant diseases.[13-16] A most recent manuscript from Locatelli et al. described a cohort of 80 patients with hematologic malignancy, all in CR, who received myeloablative conditioning that included ATG, and TCRαβ-depleted haploidentical donor progenitor cell grafts.[16] 3 year leukemia free survival was similar to HAPNK1 results at 70.7% (61-81%); TRM was similarly low and relapse rate appeared to be slightly higher at 24.3% (16-36%). Importantly, a majority received myeloablative TBI to achieve this good overall outcome. In this study then, we will utilize a similar progenitor cell graft that is selectively depleted of naïve T cells by removing alpha/beta (α/β) T cells and B cells by targeting TCRαβ and CD19 for depletion by CliniMACS.

2.4 Rationale for CD45RA-depleted DLI

As mentioned above, extensive TCD has significant negative effects on the time to donor immune competency. Donor T cells are critical for reconstituting the allogeneic host immune system, and lymphocyte recovery is an important determinate of outcome post-transplant. Transplants that employ T-cell depletion result in elimination of most memory T cells, leading to protracted immune dysfunction - an effect that becomes more severe with higher intensity conditioning regimens. This results in an increased rate of opportunistic infections. Indeed, viral infections have been a major cause of death of children receiving haploidentical transplants. The majority of these infections occur within the first 6 months following transplantation, when T-cell immunity is the lowest.

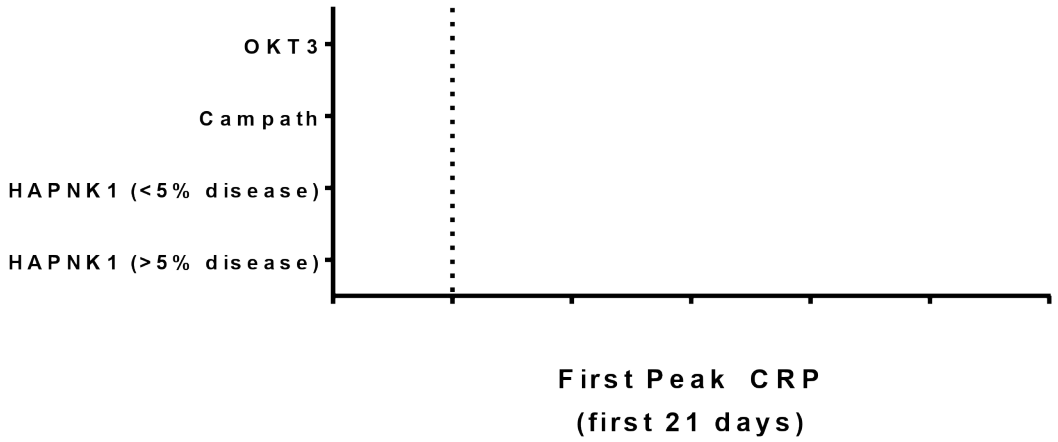
Reconstitution of immunity can be partially restored by therapeutic infusions of donor viral specific cytotoxic lymphocytes. However, in addition to requiring significant resources and expertise, the cells must be engineered and selected for specific infections and specific HLA - making this approach impractical for broad rapid application at this time. The provision of a diverse population of donor memory T cells may provide protection against several dangerous viral infections in the early post-transplant period.

The first 26 patients on HAPNK1 were analyzed for the incidence of viremia in the first 6 months after transplantation that included memory T-cell populations. Compared to the two prior haploidentical donor protocols that utilized CD3 depletion (HIFLEX and HAPREF), the incidence of CMV viremia (19.2% vs. 56.1%) and adenoviral viremia (3.8% vs 19.2%) were significantly reduced.[17] In addition, early T-cell reconstitution was markedly higher in recipients of CD45RA-depleted grafts with a median T-cell count at Day +30 of 550/uL vs 10/uL. Additional preliminary analysis as of August 2017 (HAPNK1 n=49) revealed that treatment related mortality was substantially lower in standard (non-refractory) HAPNK1 patients (green) compared to observed rates in the same patients from prior trials (black, red, blue), likely due to enhanced donor immune recovery and viral control described above.

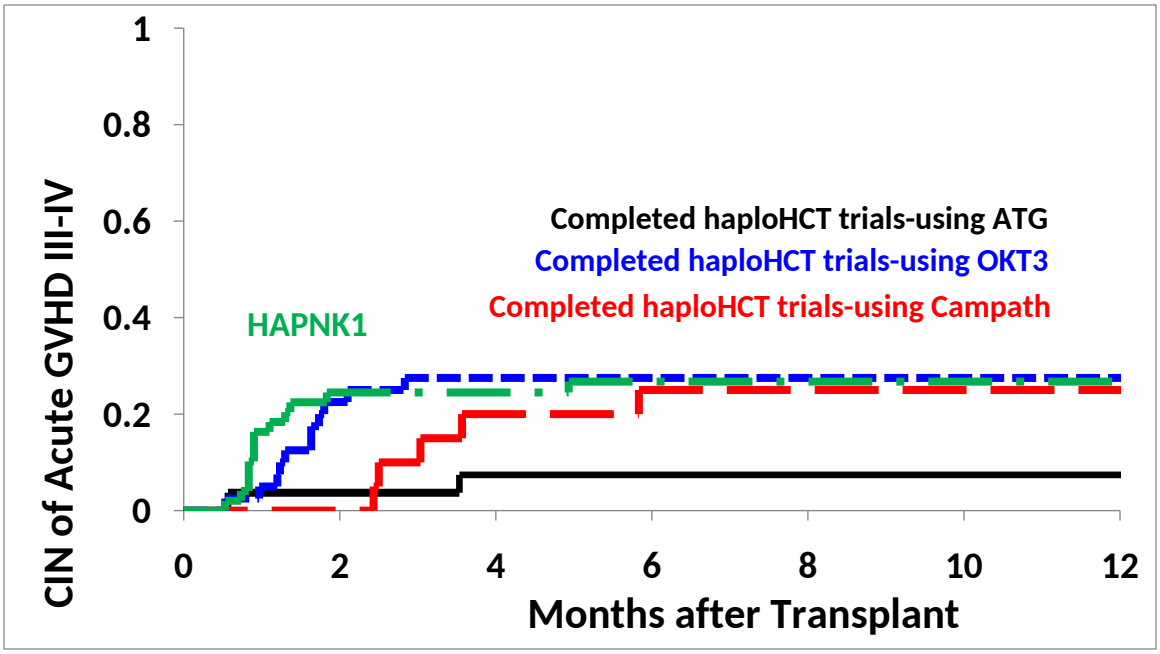


For this reason, the provision of donor CD45RA-depleted (memory) T cells will be an important part of this therapeutic protocol.

However, giving large numbers of donor memory cells with the progenitor cell graft is associated with some untoward effects. Most commonly that manifests as cytokine release syndrome. A increasingly described syndrome with T-cell therapies that variably manifests with high spiking fevers, diarrhea, liver dysfunction, acute kidney injury, fluid overload, need for noninvasive or invasive respiratory support, tachycardia with hypotension, and cardiomyopathy. Patient CRP level has been observed to correlate with CRS severity in the literature,[18] and has consistently been elevated in HAPNK1 patients compared to prior haploHCT trials as noted in the figure below.

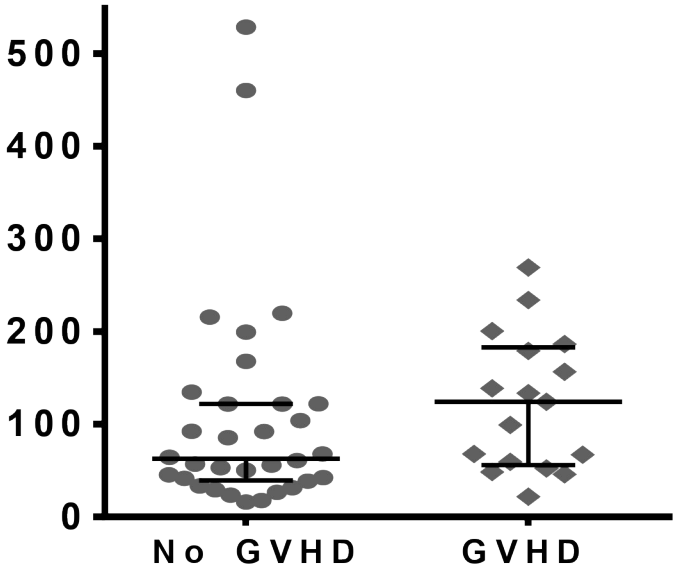


Additionally, although memory T cells are known to be less alloreactive than naïve T cells, and have demonstrated less GVHD in experimental systems, there may still be manifestations of donor memory T-cell activity in the form of GVHD. As of August 2017, the rate of severe (grade III-IV) acute GVHD has been similar on HAPNK1 (green) compared to prior haploHCT trials that utilized CD3 depletion (blue, red) despite a median T cell dose of 68 million/kg, and many recipients have received more than 100 million ($1x10^8$)/kg, whereas CD3-depletion provides a median T-cell dose less than $1x10^5$/kg (or 1000 fold less).



Although the rate of moderate to severe acute GVHD is similar, the pattern appears to be different. Specifically, 6 of the first 13 patients on HAPNK1 with grade III-IV acute GVHD manifested as isolated severe (stage 3 or 4) gut GVHD with no skin or liver involvement. When all 88 patients with acute GVHD on all haploHCT protocols were reviewed, no other patients presented with isolated severe gut GVHD. This suggests that a subset of the severe GVHD seen with donor memory cells may be driven by organ specific antigen exposure as opposed to the more a more universally expressed "self" antigen that is thought to drive GVHD in conventional HCT. Because mucositis is a nearly universal regimen related toxicity from preparative regimens of this nature, and because significant microbial antigen exposure will occur the pre-engraftment period with the absence of neutrophils and the presence of a disrupted mucosal barrier, it is plausible that delaying donor memory cell infusion until the post-engraftment period may reduce this gut specific memory T-cell GVHD phenomenon. This will also avoid the memory T cells from experiencing high inflammatory cytokine exposure that is known to occur with monocyte and neutrophil engraftment.

The infusion of conventional T cells from haploidentical donors has been associated with a significant increase in risk of acute GVHD at doses above $1x10^5$/kg. This has been seen with both DLI and with the progenitor cell graft during transplantation. With haploidentical donor memory cell infusion however, we have not seen this same kind of threshold so far. As of August 2017 (n=49) on HAPNK1, the infused T-cell dose given to patients who developed grade III-IV acute GVHD was not significantly different from the doses given to those patients who did not develop grade III-IV acute GHVD (p = 0.1 with Wilcoxon rank sum test, see figure below).

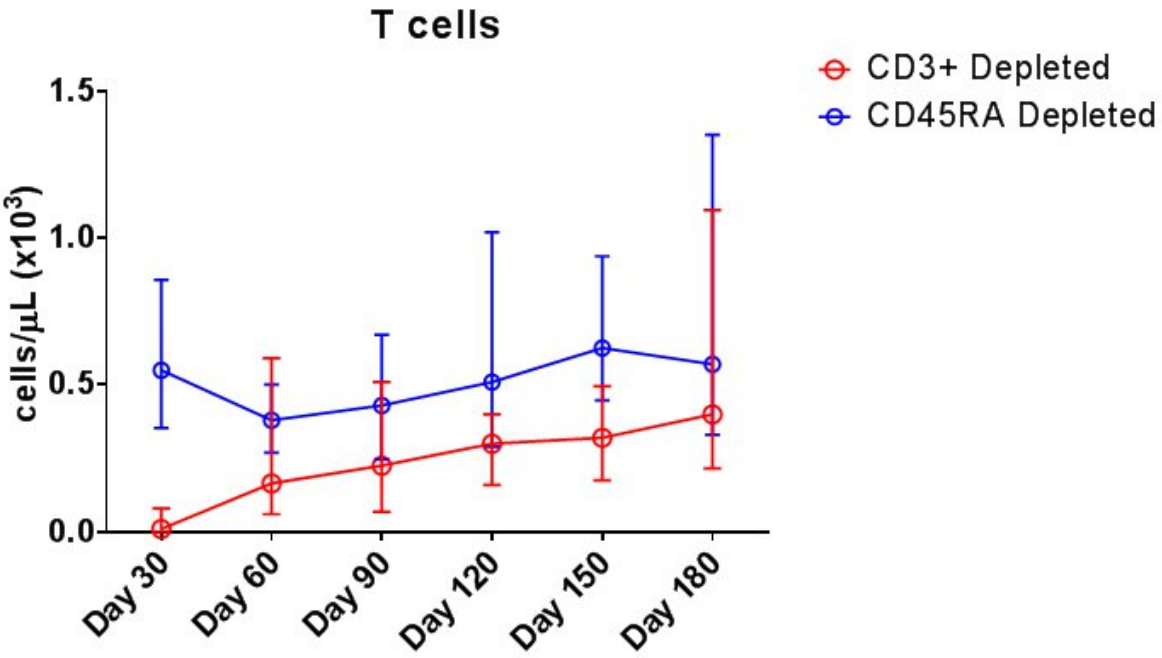


Even if there is truly no dose above which the risk of severe acute GVHD is prohibitive, perhaps the more important question is, "what is the minimally effective dose that will provide the benefits of adoptive transfer of donor memory cells?". To date, we have only anecdotal cases to explore this question. Patient #41 on HAPNK1 experienced graft rejection and was successfully salvaged with the same donor but with a CD3-depleted graft. Therefore, he did not have successful adoptive transfer of donor memory cells. His first several months were plagued with chronic infections and severe T-cell lymphopenia. Nearly 4 months after his initial transplant, he had an absolute lymphocyte count (ALC) of 100/uL and no detectible T cells. After receiving CD45RA-depleted DLI at $1x10^5$ T cells/kg, his ALC rose to 600, and his T-cell count rose to 330/uL, and he had clearance of his chronic adenovirus infection. This indicates that a dose of $1x10^5$/kg has the potential to effectively enhance donor reconstitution and protect against viral infection. Critically, this dose is known to be safe even for conventional DLI. As of July 2018, a total of 4 patients have received CD45RA-depleted DLI at doses ranging from $1x10^5$ T cells/kg to 1×10^7/kg. There has been no development of GVHD following these eight administrations of CD45RA-depleted DLI in these four patients.

Therefore, we propose to give CD45RA-depleted DLI in the early post-engraftment period, with the goal of determining the maximum effective dose as measured by memory T-cell count at 4 weeks post-DLI. (this measurement must occur any time from day 28 to day 42 after DLI, with DLI infusion as day 0). After the first 10 recipients at each dose level, if the median memory T cell count is less than 300/uL, then subsequent patients will undergo dose escalation to the next dose level (0.5 log higher). (See table below)

Dose level	
-1	Add MMF through Day +60
1	1x10^5/kg
2	1x10^6/kg
3	1x10^7/kg

Although 300/uL is somewhat arbitrary, this will allow recipeients to achieve a similar early memory T-cell recovery to patient on HAPNK1, where the median T-cell count at Day +60 was 380/uL.[17] (See figure)



2.5 Rationale for the Reduced Intensity Conditioning Regimen

The immunologic GVL effect after allogeneic HCT has allowed reduction in the intensity of conditioning, as HCT was no longer reliant purely on the cytotoxicity of high dose chemo-/radio-therapy to eradicate disease. Reduced-intensity conditioning (RIC) regimens have the potential to improve the outcomes of allogeneic HCT by decreasing the acute TRM.[19-21] Fludarabine-based RIC regimens are safe and effective alternatives to total body irradiation (TBI) in haploidentical HCT.[22-24] In addition, intensification of immunoablative conditioning using ATG, OKT3, or alemtuzumab, results in the decrease incidence of GVHD and graft rejection as well.[24-27] In our first RIC haploidentical HCT protocol REFSCT, we evaluated a RIC regimen for pediatric patients with refractory hematological malignancies or considered at high risk for TRM with a full myeloablative regimen. The

RIC regimen consisted of fludarabine (200mg/m^2), thiotepa (10mg/kg), melphalan (120 mg/m^2) and Orthoclone-OKT3 and enrolled 25 patients from 2003-2005.

Out of the 25 participants, 22 engrafted with a median of 10 days (range, 7-12) and 3 experienced graft failure. The patients with graft failure, one patient had disease relapse and 2 were salvaged with stem cells infusions from their first transplant donor. The median time to platelet engraftment to 20,000/mm^3 and 50,000/mm^3 was 17 days (range, 12-36) and 17 days (range, 12-76), respectively. The cumulative incidence of overall grade III-IV acute GVHD and chronic GVHD was 8% and 28%, respectively. Of those participants who died, 13 were due to relapsed disease (at a median of 105 days post-HCT). Only 4 (12%) died of TRM, despite most of the patients having received one prior allogeneic HCT - 1 died of GVHD, 1 of infection, 1 of hemorrhage, and 1 of cardiomyopathy. In summary, RIC allows for engraftment with an acceptable rate for graft failure, GVHD, and TRM.

Two subsequent haploidentical HCT studies at St. Jude (HAPREF and HIFLEX) also utilized non-TBI preparative regimens with a fludarabine, thiotepa, melphalan backbone. Twenty-seven patients with high risk hematologic malignancies were transplanted on one of these two protocols with OKT3 as in vivo T cell depletion, followed by infusion of a CD3$^+$ depleted graft. The one-year EFS was 51.9% ± 9.6%. These studies have demonstrated the EFS and TRM in TCD RIC HCT from mismatched haploidentical donor compare favorably with reports from MUD.

Importantly however, OKT3 was an important component of the preparative regimen for these transplants, and it is no longer commercially available. Therefore, the institutional front-line haploidentical donor HCT trial (HIFLEX) substituted Campath for OKT3. Early results indicate that survival is similar between patients on HIFLEX treated with OKT3 versus Campath (data not shown). However, requirement for the use of therapeutic DLI rose substantially. Only 1 of the 11 patients who received OKT3 on HIFLEX required more than 2 DLI in the first 100 days post-HCT. In contrast, 9 of the first 14 patients who received Campath on HIFLEX required more than 2 DLI in the first 100 days post-HCT. This is likely due to Campath's anti-NK cell effects, leading to an increased clinical need for early T-cell add back in the form of DLI. Therefore, in the subsequent protocol HAPNK1, 8 Gy total lymphoid irradiation (TLI) was used in the preparative regimen along with cyclophosphamide (60mg/kg), added to the backbone of fludarabine (150mg/m2), thiotepa (10mg/kg), and melphalan (140mg/m^2) to facilitate engraftment, and no serotherapy was utilized. As detailed above, preliminary results show donor engraftment to be reliably obtained in the early post-transplant period as graft failure occurred in 2 of 57 (3.5%) as of March 2018. Although TLI has been well tolerated in patients receiving their first HCT on HAPNK1, the heart, gut, and to a lesser extent the liver, receives significant radiation exposure with TLI. We have utilized ATG in place of TLI in our protocol for repeat allogeneic transplantation protocol (REF2HCT), because many patients will have already received significant radiation exposure, and are more heavily pre-treated than their HAPNK1 counterparts. This protocol incorporates TCRab-depleted progenitor cell graft and CD45RA-depleted progenitor cell graft, and as of March 2018 there have been 0 patients experiencing graft failure in the first 14 recipients. In addition, Locatelli et al, utilized ATG in their preparative regimens for patients with hematology malignancy who received a TCRab-depleted haploidentical donor graft, and the rate of graft failure was acceptable with 2 of 80 (2.5%) experiencing graft failure.[16] Therefore,

we hypothesize that donor engraftment can still be reliably obtained in this population without radiation if ATG is added to the chemotherapy backbone.

On this study, with the use of ATG in the preparative regimen, no additional pharmacologic acute GVHD prophylaxis will be added. The optimal dose and timing of ATG administration is unknown. Locatelli et al found that while 30% of patients experienced grade I-II acute GVDH, no single dcase of visceral GVHD or Grade III-IV acute GVHD was recorded.[16] Therefore, we will utilize the same schedule of ATG administration in this protocol. Optional PK testing will be offered to trial participants so that we can better define the exposure and duration of ATG when dosed in this manner in recipients of TCD haploidentical donor HCT. This data will allow us to optimize ATG dose and timing in future therapeutic protocols.

2.6 Rationale for Blinatumomab

Blinatumomab is a bispecific CD19-directed CD3 T-cell engager that mediates formation of a synapse between T cells and the CD19+ target cell, resulting in lysis of that CD19+ cell.[28] It was granted accelerated approval by the FDA on 12/3/2014.[28] Multiple clinical trials have demonstrated the efficacy of blinatumomab for patients with refractory B-cell malignancies.[29-31]

An early study included 21 patients with refractory disease at an minimal residual disease level of burden. Blinatumomab dosing was 15μg/m2/day for 28 days, and 16 of 20 evaluable patients responded by becoming MRD negative.[29] Fevers were common, but there was no mention of cytokine release syndrome (CRS), and one patient experienced a seizure that was reversible. Following an initial dip, T-cell numbers quickly returned to baseline consistent with T-cell re-distribution, and then 19 of the 20 patients who completed therapy had a significant increase in T-cell numbers at a median of 17 days into treatment.[32] The contribution of this T-cell expansion was predominately from the effector memory T-cell ($T_{EM)}$ fraction.[29,32]

This same group also treated 36 adults with active disease (>5%), and saw 25 responders amongst 36 adult patients.[30] That includes 8 responders amonst the 15 patients who relapsed after a prior allogeneic HCT. Fever was again common (>80%), and several cases of more severe CRS were noted. Two grade 4 CRS were associated with a disease burden in excess of 50% blasts in the marrow. A dose finding portion of this study determined that starting at 15μg/m2/day was associated with a high rate of adverse events (and serious advese events), and the preferred dose was ultimately chosen to be 5μg/m2/day x 1 week, followed by 15μg/m2/day x 3 weeks, to comprise a full 28 day course.[30] Also noted was 6 patients with temporary or permanent discontinuation of blinatumomab for neurologic or psychiatric adverse events, all of which resolved within 72 hours. 9 responders subsequently received allogeneic HCT as consolidative therapy, and toxicity was no greater than expected following blinatumomab. The authors recommend HCT as standard of care post-blinatumomab if response obtained. Long term follow-up of these patients confirmed that long term survivors had a more pronounced T-cell expansion, and that the expansion was predominantly T_{EM}; whereas non responders had the lowest T-cell and T_{EM} counts.[33] They also noted that patients who failed to respond with the first course, were unlikely to respond after a second course.

Recently, a large multicenter phase 2 trial in which 189 adults with relapsed/refractory B cell malignancies received blinatumomab was published.[31] Dosing was in graduated fashion, with a lower dose the first week. The overall response rate was 43%, and was 45% in the 64 patients with a prior allogeneic HCT.

The response rate in those with less than 50% blasts in their bone marrow was 73%. With dexamethasone pre-treatment in all patients, only 3 (2%) experienced grade 3 CRS, though neurologic toxicities were more common with 11% grade 3 and 2% grade 4 events. Tremor, dizziness, and confusion were most common neurologic events.

There is also 9 additional patients in the literature who have been given blinatumomab for post-transplant relapse.[34] 4 of the 9 achieved CR after a single course. Two who failed to respond to a first course at 5μg/m2/day, did respond to a second course at 15μg/m2/day; 3 had no response. There was no difference in the starting T-cell numbers in responders and non-responders, though both were generally under 200 T-cells/μL despite being a median of over 8 months post-transplant.[34] Fever, fatigue, and drop in blood pressure were common, except for one non-responder and 1 patient with minimal disease burden. They found that T-cell to blast ratio was important, and patients who did the best had a relatively high number of T cells and relatively low disease burden.[34]

There is a strong rationale for the addition of blinatumomab for patients with CD19+ hematologic malignancy in this trial. Firstly, prognosis is uniformly poor in patients who relapse after allogeneic HCT, and therefore any therapy that has the potential to reduce postHCT relapse has the potential to increase long term survival. Additionally, blinatumomab has demonstrable efficacy in patients with chemotherapy refractory B-cell malignancies, and it has efficacy in the post-transplant setting. Blinatumomab appears to work best when a low disease burden is present, and this is likely to be the case in the early post-transplant period. It also appears to need a minimum host T-cell presence, with preference for the presence of T_{EM} cells. In HAPNK1, which also utilized CD45RA depletion, there was a robust early recovery of T cells, with 11 of the first 16 patients having normal CD8+ T-cell numbers at Day +30.[35] An abundance of both effector and central memory T cells was also noted at Day +30 in HAPNK1 recipients confirming successful adoptive transfer. Therefore, the good blinitumomab activity would be predicted to be present early after CD45RA-depletion DLI due to the adoptive transfer of large numbers of donor memory T cells. Finally, memory T cells have documented GVL effects (see section 2.4), however their specificity will be MHC restricted, as demonstrated by 2 of the first 4 relapses on HAPNK1 showing haplotype loss.[36] Blinatumomab provides a direct antigen recognition that is not MHC restricted and does not require T cells that recognize leukemia or patient specific peptides, although antigen specific therapy to CD19 can likewise be circumvented by antigen loss.[37] The combination of donor memory cells and blinatumomab however could provide graft-versus-malignancy effects in both MHC restricted and whole antigen specific mechanisms, which may reduce mutational escape.

Combinatorial immune therapy is likely to be a major component of future cancer therapy. Bispecific T-cell Engager (BiTE) are currently in development for AML and multiple solid tumors.[38-41] Therefore, successful incorporation of a BiTE such as blinatumomab into HCT that provides donor memory cells may provide a robust platform for additional diseases.

For Amendment 2, the minimum peripheral blood CD3+ count to receive blinatumomab will be set at 150/μL, and patients must be at least 28 days (4 weeks) following their CD45RA-depleted (memory T-cell) DLI. These new parameters are being added due to the experience with the first 16 patients to receive blinatumomab on this protocol.

None of the 4 patients who had a CD3+ count less than 150/μL at the initiation of the blinatumomab infusion experienced response (B cell depletion). However, 11 of the 12 patients with a CD3+ count of 150/μL or greater showed effective depletion of circulating B cells. In addition, none of the 3 patients who initiated blinatumomab at less than 28 days from the protocol mandated memory T-cell DLI showed a response.

2.7 Minimal Residual Disease

Detection of minimal residual disease (MRD) prior to HCT can have a significant impact on prognosis.[42] Detection of leukemic cells that are below the limits of detection by standard morphologic examination allow early interventions when the patients are MRD positive but still in remission. Therefore, detection of MRD following transplant and early intervention may improve post-transplant clinical outcome. For these reasons, the testing of MRD prior to transplantation and as a part of disease evaluations post-transplantation is now an established standard clinical procedure.

2.8 Rationale for Present Study

For many patients with high risk hematologic malignancies that have a poor prognosis with conventional chemotherapy, allogeneic HCT offers an increased chance of cure, yet is fraught with several hazards.

HCT utilizing a related haploidentical donor offers a unique advantage in that it can be performed quickly, the donor is readily available and highly motivated, the degree of HLA mismatching may provide stronger graft-vs.-leukemia effects (GVL), and accessibility of the donor facilitates post-transplant cellular therapeutics such as donor lymphocyte infusion (DLI). Memory T cells and TCRγδ T cells have demonstrated antileukemic effects, and may provide useful anti-leukemic effects in the early post-transplant phase. Furthermore, for this often heavily pretreated patient population, it is important to explore new regimens using novel conditioning components and novel GVHD prevention strategies to take advantage of the immunologic anti-cancer effects of allogeneic HCT and to decrease transplant-related toxicity and mortality.

In this study, we will build upon the improved outcomes seen with the use of CD45RA-depletion of donor progenitor cell grafts in HAPNK1, by attempting to identify the lowest effective dose of haploidentical donor memory T cells in the early post-transplant setting. We hypothesize that this will allow us to maintain the improvement in efficacy through retention of the benefits of donor memory cells such as enhanced viral control, reduction in therapy related mortality, and possibly reduction in relapse; while simultaneously reducing the overall toxicity of the HAPNK1 regimen by giving the memory cells after engraftment and avoiding radiation therapy in the preparative regimen. We will also assess the safety and tolerability of the addition of blinatumomab in those patients with CD19+ malignancies. At the same time, we will describe the regimen-related toxicities,

lymphohematopoietic reconstitution, graft failure, and incidence of acute and chronic GvHD.

2.9 Rationale for Early DLI Cohort in Amendment 5

Following the dose escalation period of this study in which 29 patients received escalating doses of CD45RA-depleted DLI per protocol (Dose level 1 n=9; Dose level 2 n=10; Dose level 3 n=10), the maximum effective dose was determined to be Dose level 3 = 1x10^7/kg. The infusion of the memory DLI were well tolerated, and one month post infusion, there was a significant increase in the median number of CD3 T-cells, including CD8 and CD45RO+ T-cell subsets (**p<0.01 and ***p<0.001, Fig 1A). There was also significant expansion of virus-specific T-cells (VSTs) directed towards Cytomegalovirus (CMV), Adenovirus (AdV), BK, or HHV-6 as shown by Elispot assays (**p<0.01, Fig. 1B).[43]

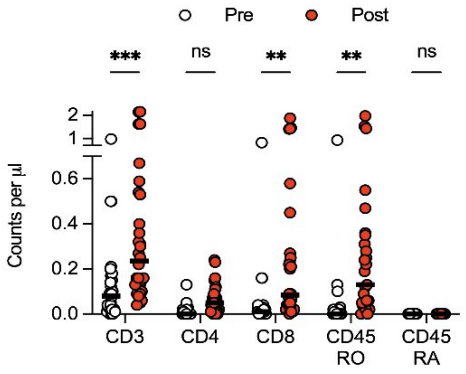


Fig 1A: Phenotypic immune reconstitution following CD45RA depleted T-cell addback: Significant increase in the median number of CD3 T-cells including CD8 and CD45RO+ T-cell subsets (**p<0.01, ***p<0.001) before and 1 month after infusion.

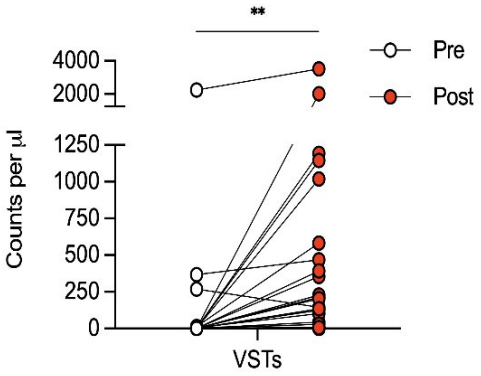


Fig 1B: Functional immune reconstitution following CD45RA depleted T-cell addback: Significant expansion of virus-specific T-cells (VSTs) directed towards CMV, Adv, BK, or HHV-6 as judged by ELispot assays before and 1 month after infusion (**p<0.01).

While this robust donor memory cell recovery appeared to shorten the duration for those patients who experienced viremia, 29 of 50 (58%) patients still experienced viremia (CMV/EBV/adenovirus). Evaluation of all patients who received memory DLI per protocol found that the median day of DLI infusion was Day +28.5 and the mean day of infusion was Day +32 (min 22, max 57). This means that the robust expansion of donor memory T-cells noted above typically did not occur until about two months following transplant, the time period when patients are most at risk of serious transplant related complications. The primary goal of this Amendment 5 expansion (early DLI) cohort then is to move up (closer to the date of transplant) the donor memory DLI infusion by two weeks.

To accomplish this goal, the preparative regimen will have one alteration – the doses of ATG will be moved back (away from the date of transplant) by 7 days. Instead of ATG infusions on Day -5, -4, and -3 the infusions will instead occur on Day -12, -11, and -10. (See section 4.1.2) This will minimize any potential ATG effects on the early infusion of donor memory cells.

The goal day of memory DLI infusion at the determined MED of 1x10^7/kg then will be around Day +14, with the goal of most (if not all) infusions to occur on Day +10 to Day +21. Infusions after Day +21 are allowed if the patient was otherwise not suitable for infusion prior to Day +21.

3.0 RESEARCH PARTICIPANT ELIGIBILITY CRITERIA AND STUDY ENROLLMENT

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

3.1 Inclusion Criteria for Transplant Recipient

3.1.1 Age less than or equal to 21 years.

3.1.2 Does not have a suitable HLA-matched sibling donor (MSD) or volunteer 10/10 HLA-matched unrelated donor (MUD) available in the necessary time for progenitor cell donation.

3.1.3 Has a suitable single haplotype matched (≥ 4 of 8) family member donor.

3.1.4 High risk hematologic malignancy.

3.1.4.1 High risk ALL in CR1.

Examples include, but not limited to: t(9;22) with persistent or recurrent transcript, hypodiploid cytogenetics, MRD >1% at the end of induction, M2 or greater marrow at the end of induction, recurrent or rising MRD after induction, Infants with MLL fusion or t(4;11), relapse after prior CART therapy.

3.1.4.2 ALL in High risk CR2.

Examples include, but not limited to t(9;22), BM relapse <36 mo CR1 or <6mo after completion of therapy, any T-ALL, very early (< 6mo CR1) isolated CNS relapse, late BM relapse with poor response to standard reinduction therapy(e.g. MRD positive or recurrence after two blocks), relapse after prior CART therapy.

3.1.4.3 ALL in CR3 or subsequent.

3.1.4.4 AML in high risk CR1 (diagnosis of AML includes myeloid sarcoma).

Examples include but not limited to: preceding MDS or MDS-related AML, FAB M0, FAB M6, FAB M7 with high risk genetics such as ML not t(1;22), MRD > 0.1% after two cycles of induction, MRD > 1% after one cycle of induction, FLT3-ITD in combination with NUP98-NSD1 fusion or WT1 mutation, any high risk cytogenetics such as: DEK-NUP214 [t(6;9)], KAT6A-CREBBP [t(8;16)], RUNX1-CBFA2T3 [t(16;21)], -7, -5, 5q-, KMT2A-MLLT10 [t(6;11)], KMT2A-MLLT4 [t(10;11)], inv(3)(q21q26.2), CBFA2T3-GLIS2 [inv(16)(p13.3q24.3)], NUP98-KDM5A [t(11;12)(p15;p13)], ETV6-HLXB [t(7;12)(q36;p13)], NUP98-HOXA9 [t(7;11)(p15.4;p15)], NUP98-NSD1.

3.1.4.5 AML in CR2 or subsequent.

3.1.4.6 Therapy related AML, with prior malignancy in CR > 12mo

3.1.4.7 MDS, primary or secondary

3.1.4.8 NK cell, biphenotypic, or undifferentiated leukemia/lymphoma in CR1 or subsequent.

3.1.4.9 CML in accelerated phase, or in chronic phase with persistent molecular positivity or intolerance to tyrosine kinase inhibitor.

3.1.4.10 Hodgkin lymphoma in CR2 or subsequent after failure of prior autologous HCT, or unable to mobilize progenitor cells for autologous HCT.

3.1.4.11 Non-Hodgkin lymphoma in CR2 or subsequent after failure of prior autologous HCT, or unable to mobilize progenitor cells for autologous HCT.

3.1.4.12 JMML

3.1.5 If prior CNS leukemia, it must be treated and in CNS CR

3.1.6 Does not have any other active malignancy other than the one for which this HCT is indicated.

3.1.7 No prior allogeneic HCT, and no autologous HCT within the previous 12 months.

3.1.8 Patient must fulfill pre-transplant organ function criteria:

3.1.8.1 Left ventricular ejection fraction > 40%, or shortening fraction ≥ 25%.

3.1.8.2 Creatinine clearance (CrCl) or glomerular filtration rate (GFR) ≥ 50 ml/min/1.73m^2.

3.1.8.3 Forced vital capacity (FVC) ≥ 50% of predicted value; or pulse oximetry ≥ 92% on room air if patient is unable to perform pulmonary function testing.

3.1.8.4 Karnofsky or Lansky (age-dependent) performance score ≥ 50 (See APPENDIX A).

3.1.8.5 Bilirubin ≤ 3 times the upper limit of normal for age.

3.1.8.6 Alanine aminotransferase (ALT) or Aspartate aminotransferase (AST) ≤ 5 times the upper limit of normal for age.

3.1.8.7 Not pregnant. If female with child bearing potential, must be confirmed by negative serum or urine pregnancy test within 14 days prior to enrollment.

3.1.8.8 Not breast feeding

3.1.8.9 Does not have current uncontrolled bacterial, fungal, or viral infection.

3.2 Inclusion Criteria for Haploidentical Donor

3.2.1 At least single haplotype matched (≥ 4 of 8) family member

3.2.2 At least 18 years of age.

3.2.3 HIV negative.

3.2.4 Not pregnant as confirmed by negative serum or urine pregnancy test within 14 days prior to enrollment (if female).

3.2.5 Not breast feeding.

3.2.6 Regarding donation eligibility, is identified as either:

3.2.6.1 Completed the process of donor eligibility determination as outlined in 21 CFR 1271 and agency guidance; OR

3.2.6.2 Does not meet 21 CFR 1271 eligibility requirements, but has a declaration of urgent medical need completed by the principal investigator or physician sub-investigator per 21 CFR 1271.

3.3 Research Participant Recruitment and Screening

This study will be posted on http://clinicaltrials.gov. Potential transplant recipients are typically referred from their primary clinical service at St. Jude, from the Physician Referral Office for non-St. Jude patients, or are patients currently being treated by the Department of BMTCT. Each patient considered for transplantation is registered on the "Transplant List," an ongoing list used to provide the clinical service with information regarding new patients for consultation and under consideration for transplantation and the status of those undergoing pre-evaluation. The patient remains on this list until a decision has been confirmed for acceptance or rejection based on several factors, primarily completion of prior therapy and/or clinical status. The proposed patient schedules on this form are often subject to change due to various reasons such as family travel issues, donor availability for procurement of progenitor cells, recipient clinical status, etc. This list is a confidential document, updated and maintained by the Department of BMTCT Coordinators.

3.4 Enrollment on Study at St. Jude

A member of the study team will confirm potential participant eligibility as defined in Section 3.1 - 3.2, and register the participant in OnCore. A research participant-specific consent form and assent document (where applicable) will be generated. The entire signed consent/assent form(s) must be scanned into the Electronic Health Record (EHR) by the study team designee.

4.0 TREATMENT PLAN

4.1.1 Preparative regimen for original cohort (through Amendment 4)

DAY	MEDICATION	DOSE	DOSE #
-9	Cyclophosphamide	60 mg/kg intravenous once daily	1 of 1
-8	Fludarabine*	30 mg/m^2 intravenous once daily	1 of 5
-7	Fludarabine*	30 mg/m^2 intravenous once daily	2 of 5
-6	Fludarabine*	30 mg/m^2 intravenous once daily	3 of 5
-5	Fludarabine*	30 mg/m^2 intravenous once daily	4 of 5
	ATG (rabbit)	1mg/kg intravenous daily	1 of 1
-4	Fludarabine*	30 mg/m^2 intravenous once daily	5 of 5
	ATG (rabbit)	2mg/kg intravenous daily	1 of 2
-3	Thiotepa	5 mg/kg intravenous twice daily	1,2 of 2
	ATG (rabbit)	2mg/kg intravenous daily	2 of 2
-2	Melphalan**	70 mg/m^2 intravenous once daily	1 of 2
-1	Melphalan**	70 mg/m^2 intravenous once daily	2 of 2
0	HPC,A Infusion(TCRα/β+ and CD19+ depleted)		
+1	HPC,A infusion (if needed to achieve goal CD34+ cell dose, see section 4.5)		
+2-5	--		
+6	G-CSF	5mcg/kg subcutaneous or intravenous daily until ANC >2000 for 2 consecutive days, or as clinically indicated	
TBD¶	CD45RA-depleted DLI	Dose based on dose level (See section 2.4 and 4.7)	
TBD‡	Blinatumomab	5mcg/m2/day x 7days, then 15mcg/m2/day x 21days	

* Fludarabine dosing will be 1 mg/kg/day for patients ≤ 10kg
** Melphalan dosing will be 2.3 mg/kg/day for patients ≤ 10kg
¶ CD45RA-depleted DLI will be given at least two weeks after engraftment (see section 4.7)
‡ Blinatumomab will be given at least four weeks post-DLI, and only to patients with CD19+ malignancies, and only once the peripheral blood CD3+ count is at least 150/μL. (see section 4.2)

4.1.2 Preparative regimen for early DLI cohort (Amendment 5)

DAY	MEDICATION	DOSE	DOSE #
-12	ATG (rabbit)	1mg/kg intravenous daily	1 of 1
-11	ATG (rabbit)	2mg/kg intravenous daily	1 of 2
-10	ATG (rabbit)	2mg/kg intravenous daily	2 of 2
-9	Cyclophosphamide	60 mg/kg intravenous once daily	1 of 1
-8	Fludarabine*	30 mg/m^2 intravenous once daily	1 of 5
-7	Fludarabine*	30 mg/m^2 intravenous once daily	2 of 5
-6	Fludarabine*	30 mg/m^2 intravenous once daily	3 of 5
-5	Fludarabine*	30 mg/m^2 intravenous once daily	4 of 5
-4	Fludarabine*	30 mg/m^2 intravenous once daily	5 of 5
-3	Thiotepa	5 mg/kg intravenous twice daily	1,2 of 2
-2	Melphalan**	70 mg/m^2 intravenous once daily	1 of 2
-1	Melphalan**	70 mg/m^2 intravenous once daily	2 of 2
0	HPC,A Infusion(TCRα/β+ and CD19+ depleted)		
+1	HPC,A infusion (if needed to achieve goal CD34+ cell dose, see section 4.5)		
+2-5	--		
+6	G-CSF	5mcg/kg subcutaneous or intravenous daily until ANC >2000 for 2 consecutive days, or as clinically indicated	
+10-21	CD45RA-depleted DLI	1x10^7 CD3+ cells/kg ¶	
TBD‡	Blinatumomab	5mcg/m2/day x 7days, then 15mcg/m2/day x 21days	

* Fludarabine dosing will be 1 mg/kg/day for patients ≤ 10kg

** Melphalan dosing will be 2.3 mg/kg/day for patients ≤ 10kg

¶ CD45RA-depleted DLI will be given around Day +14 (goal range Day 10 to 21)

‡ Blinatumomab will be given at least four weeks post-DLI, and only to patients with CD19+ malignancies, and only once the peripheral blood CD3+ count is at least 150/μL. (see section 4.2)

Cellular infusion procedures and monitoring

For the proper infusion procedures and monitoring of the HPC product please refer to BMTCT SOP 40.02 “Hematopoietic Progenitor Cell Infusion – FRESH (Allogeneic): IV Push and IV Drip” or SOP 40.03 “Hematopoietic Progenitor Cell Infusion – FROZEN: IV Push”. Please note that all relevant SOPs can be found on the BMTCT Clinical Transplant Program intranet page.

Importantly, during the cellular infusions, monitoring of vital signs, breath sounds, heart rate, pulse oximetry, and I/O will be done per the established transplant nursing procedure, as well as appropriate Department of BMTCT SOPs, then documented on the Cellular Product Infusion Record. If a reaction is suspected at any time during the infusion, the nurse will 1. Stop the infusion, 2. Notify the Attending Transplant Physician immediately, 3. NOT discard the product until physician orders are given. Proper documentation (symptoms of patient, vital signs, actions taken, outcome, and follow-up) will be completed in the Cellular Product Infusion Record.

General treatment related comments:

- The Hematopoietic Progenitor Cell, Apheresis (HPC,A) infusion(s) may be delayed by approximately 24 hours in order to accommodate progenitor cell collection with the donor, the Blood Donor Center and/or HAL as well as the research participant clinical condition.

- The term “every” used in tables is an approximate term meaning that these medications noted will be administered approximately “every” 12 hours. The drug administration timing in the case of “every 12 hours” may be modified by approximately +/- 4 hours or as clinically indicated such as in the case of surgical procedures or to accommodate other necessary medication, blood product delivery, or procedures (such as a needed CT scan). The term “day” does not refer to an absolute calendar day. It refers to a general 24-hour period.

- Dosing for the medications cyclophosphamide, fludarabine, thiotepa, and melphalan may be modified for research recipients based upon actual body weight or adjusted ideal body weight when clinically indicated. Mesna will be administered for prevention of hemorrhagic cystitis from the medication cyclophosphamide. In general, mesna is administered at 15 mg/kg/dose prior to cyclophosphamide and at approximately 3, 6, and 9 hours after the cyclophosphamide infusion, to give a 1:1 ratio of mesna:cyclophosphamide. Mesna dose and administration schedule may vary based on physician recommendation.

- Criteria for medication calculations based on body weight/body surface area and other medication related information can be found in the St. Jude Formulary or the St. Jude Dept of Pharmaceutical Sciences intranet website. Medication doses may be rounded to the nearest integer or to the nearest appropriate quantity when clinically or pharmaceutically indicated as per the MD and PharmD.

- This treatment plan includes ATG in the preparative regimen and selective T-cell depletion for GVHD prophylaxis. No post-transplant pharmacologic GVHD prophylaxis will be routinely given. In the event the participant develops GVHD, treatment will be according to the SOPs of the St. Jude Department of BMT&CT.

4.2 Blinatumomab Administration

Blinatumomab will be given to patients with a history of CD19+ malignancy as determined by St. Jude hematopathologist review of current and historical specimens and reports. The PI had a personal patient (unpublished) that received CD19+ directed therapy, and developed CD19 negative leukemia (by flow cytometry) and was then transplanted, and relapsed with CD19+ disease again. Therefore, patients who were previously CD19+ and became CD19 negative after antigen specific therapy, will still be offered blinatumomab at the discretion of their primary transplant physician after a discussion of the risks and benefits.

Blinatumomab dosing will begin no sooner than 4 weeks after CD45RA-depleted DLI and no later than Day +180. There must be no acute GVHD or it must be quiescent. ALT must be less than 5x ULN, bili less than or equal to 1.5x ULN, and creatinine less than or equal to 1.5x ULN. Tylenol and/or Benadryl may be given as pre-medication. Dexamethasone will not routinely be given as pre-medication, but its use is not prohibited.

Dosing will be 5mcg/m2/day x 7days, then 15mcg/m2/day x 21days. Dosing will be based on actual BSA with no dose adjustment for obese patients. For patients greater than or equal to 45kg, dosing will be 9mcg/day x 7 days, then 28mcg/day x 21 days.

Blinitumomab infusion will be held for Grade 3 neurologic toxicity or any other relevant Grade 3 or 4 toxicity. Missed days will not be made up by adding extra days to the originally planned 28 day course. If the interruption is greater than 14 days, blinatumomab will be permanently discontinued. Blinatumomab may also be discontinued for disease progression despite blinatumomab, or if continued administration is not deemed beneficial in the investigator's opinion. Specific recommendations for withholding, permanently discontinuing, or dose alteration of blinatumomab due to toxicity are in the chart that follows:

Toxicity	Grade*	Patients Greater Than or Equal to 45 kg	Patients Less Than 45 kg
Cytokine Release Syndrome (CRS)	Grade 3	Withhold BLINCYTO until resolved, then restart BLINCYTO at 9 mcg/day. Escalate to 28 mcg/day after 7 days if the toxicity does not recur.	Withhold BLINCYTO until resolved, then restart BLINCYTO at 5 mcg/m^2/day. Escalate to 15 mcg/m^2/day after 7 days if the toxicity does not recur.
	Grade 4	Discontinue BLINCYTO permanently.	
Neurological Toxicity	Seizure	Discontinue BLINCYTO permanently if more than one seizure occurs.	
	Grade 3	Withhold BLINCYTO until no more than Grade 1 (mild) and for at least 3 days, then restart BLINCYTO at 9 mcg/day. Escalate to 28 mcg/day after 7 days if the toxicity does not recur. If the toxicity occurred at 9 mcg/day, or if the toxicity takes more than 7 days to resolve, discontinue BLINCYTO permanently.	Withhold BLINCYTO until no more than Grade 1 (mild) and for at least 3 days, then restart BLINCYTO at 5 mcg/m^2/day. Escalate to 15 mcg/m^2/day after 7 days if the toxicity does not recur. If the toxicity occurred at 5 mcg/m^2/day, or if the toxicity takes more than 7 days to resolve, discontinue BLINCYTO permanently.
	Grade 4	Discontinue BLINCYTO permanently.	
Other Clinically Relevant Adverse Reactions	Grade 3	Withhold BLINCYTO until no more than Grade 1 (mild), then restart BLINCYTO at 9 mcg/day. Escalate to 28 mcg/day after 7 days if the toxicity does not recur. If the toxicity takes more than 14 days to resolve, discontinue BLINCYTO permanently.	Withhold BLINCYTO until no more than Grade 1 (mild), then restart BLINCYTO at 5 mcg/m^2/day. Escalate to 15 mcg/m^2/day after 7 days if the toxicity does not recur. If the toxicity takes more than 14 days to resolve, discontinue BLINCYTO permanently.
	Grade 4	Consider discontinuing BLINCYTO permanently.	

*Based on the Common Terminology Criteria for Adverse Events (CTCAE). Grade 3 is severe, and Grade 4 is life-threatening.

The median onset for cytokine release syndrome (CRS) is 2 days[33], therefore patients will be admitted to the hospital for a minimum of 72 hours for the initial dosing of Blinatumomab. Dexamethasone does not inhibit cytotoxic activity of BiTE activated T cells, and therefore it may be given for patients who experience high grade CRS.[44] However, it can be generally immune suppressive and lympholytic, therefore an IL-6 inhibitor such as tocilizumab is the preferred first agent for CRS.[45]

As described in Section 11.1, if two patients in the first six experience permanent discontinuation of blinatumomab (as described above), then the study will be placed on hold to further blinatumomab administration; and protocol modification will occur to adjust dosing and premedication, or to remove blinatumomab administration. Because the use of blinatumomab at this time point is novel and the safety is unknown, the provision of blinatumomab to the first six qualified recipients will be staggered. If one patient is on blinatumomab, a second patient cannot start blinatumomab therapy until the prior patient has received at least 72 hours at the second dose level (i.e. after 10 days total blinatumomab dosing) without interruption. If the prior patient experiences interruption, then the next patient cannot begin blinatumomab until the prior patient successfully completes the four

week course. No more than two patients may receive blinatumomab at the same time. Once one permanent discontinuation has occurred, only one patient is allowed to receive blinatumomab at a time. (i.e. after one permanent discontinuation for safety reasons, each prior patient must successfully complete blinatumomab dosing before a subsequent patient can initiate blinatumomab.)

This sequential step-wise approach prevents more than two patients in the first six from experiencing blinatumomab toxicity of a severity that requires permanent discontinuation. It also allows a second patient to initiate blinatumomab without waiting for the prior patient to complete their course if no prior permanent discontinuation has occurred. Because protocol accrual is anticipated to be approximately 10 recipients per year, and because about 33% of recipients will be CD19+ (and therefore qualify for blinatumomab), significant overlap that would trigger this staggering rule is expected to be rare.

It is the treatment with blinatumomab that will be staggered and not enrollment onto the protocol. Many patients enrolled on this protocol have limited other potentially curative therapy available and will have a short time period during which they are suitable transplantation (due to disease or clinical condition factors). To delay or reschedule a HCT due to waiting for another patient to complete blinatumomab places significant risk on prospective patients who have the potential to benefit from this transplantation therapy, even if blinatumomab may not be available. Furthermore, if a patient were not to receive blinatumomab, the patient would still be evaluable for other study endpoints including the primary objective.

4.3Donor Selection

If more than one family member donor is suitable, then donor selection will be based on the preference of the primary transplant attending. Factors in selection will include degree of KIR mismatching, donor-recipient matching of CMV serology, donor-recipient red blood cell compatibility, degree of HLA matching, size of the potential donor, previous use as a donor, presence of donor-specific antibody, and overall health and availability of the potential donor.

Donor eligibility for cell collection will be determined through the guidelines outlined in 21 CFR 1271 and the Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps). Potential donors will undergo an initial screening process that will include at least a complete physical exam, history and testing for relevant communicable diseases. Physical exams to evaluate donor candidacy will be conducted by a non-Department of BMTCT physician

(St. Jude or non-St. Jude). For subsequent therapeutic cell collection procedures, if a complete screening procedure has been performed within the previous 6 months, an abbreviated donor screening procedure may be used for these repeat donations. The abbreviated screening procedure must determine and document any changes in the donor's medical history since the previous donation that would make the donor ineligible, including changes in relevant social behavior.

If a donor is determined to be ineligible, the donor is not automatically excluded. Part 21 CFR 1271.65 (b)(1)(i) allows use of ineligible donors who are first or second degree blood relatives. In this situation, the physician will document the necessity of using the ineligible donor by providing a statement of "Urgent Medical Need" as explained in the 21 CFR

1271.3 (u). The cell therapy products will be labeled as required in 21 CFR 1271.65 (b)(2). Recipients or their legal guardians will be informed of the use of an ineligible donor.

Please see Departmental SOP 30.05 "Determination of Eligibility and Suitability for Progenitor Cell and Lymphocyte Cell Donors" for additional information. Please note that all relevant SOPs can be found on the BMTCT Clinical Transplant Program intranet page.

4.4Donor Mobilization and Graft Collections

A G-CSF (or biosimilar) mobilized peripheral blood progenitor cell product (identified as HPC,A) is the preferred progenitor cell graft source. Our desired target goal will be 5 x 10^6 $CD34^+$ cells/kg. This number of cells will be necessary to provide an adequate graft, following the various ex vivo manipulations, for prompt reconstitution. More then one collection may be needed to achieve this goal. Donors will undergo a standard hematopoietic progenitor cell mobilization regimen consisting of 5 days of G-CSF given subcutaneously at 10 micrograms/kilogram. The graft will be collected by leukapheresis on day 5 (and 6 if needed) of G-CSF. The HPC product will typically be collected and infused fresh, however there may be patients or logistical situations that require the HPC product to be collected early, processed, and stored frozen.

The decision to use a fresh versus frozen HPC will be made by the PI and/or primary transplant attending based on patient and donor factors, as well as potential scheduling conflicts.

DONOR MOBILIZATION TIME LINE (if fresh HPC product)		
DAYS	**MEDICATION**	**APHERESIS**
Day -5	G-CSF* 10 mcg/kg/day SC**	
Day -4	G-CSF* 10 mcg/kg/day SC**	
Day -3	G-CSF* 10 mcg/kg/day SC**	
Day -2	G-CSF* 10 mcg/kg/day SC**	
Day -1	G-CSF* 10 mcg/kg/day SC**	Apheresis for HPC graft
Day 0	G-CSF* 10 mcg/kg/day SC** (if needed)	Apheresis for HPC graft (if needed)

*Or biosimilar

** G-CSF may be reduced if the donor's WBC is >75.0 x 10^6/mL

The dose of G-CSF may require modification based on the complete blood counts (CBC). If the donor's white blood count (WBC) is >75.0 x 10^6/ml the dose of cytokine administered will be reduced. The guidelines for dose modification can be found in the St.

Jude Children's Research Hospital Department of BMT and CT SOP 30.06.00 “The practice for the evaluation, preparation and care of allogeneic and autologous donors mobilized with growth factor.” Please note that all relevant SOPs can be found on the BMTCT Clinical Transplant Program intranet page.

The daily leukapheresed volumes for HPC,A collection is generally 3–4 total blood volumes. Two additional days of leukapheresis may be performed at the physician’s discretion (no more than 3 total) to reach the cell dose target, however, this is expected to be rare.
Leukapheresis may be terminated early upon request of donor, or when deemed medically necessary per the judgment of the treating sub-investigator physician or principal investigator. All products will be collected as per Foundation for the Accreditation of Cellular Therapy (FACT) and AABB guidelines. Donors will be monitored during the period of the mobilization and leukapheresis procedure for adverse events (Appendix C).

If we are unable to collect the minimum dose of 2 x 10^6 $CD34^+$ cells/kg of recipient weight from the first donor, then an alternative family member may be used if he/she fulfills all the donor criteria described in section 3.2.

4.5 Progenitor Cell Graft Preparation

Graft evaluation and preparation will take place in the Human Applications Laboratory (HAL) in the Department of BMTCT using established SOP.

The HPC product(s) will be TCD using the investigational CliniMACS device and TCRαβ Biotin reagent and CD19 reagent, and tubing set as directed by the manufacturer (Miltenyi Biotech). See section 5.2 for additional CliniMACS device information. Briefly, hematopoietic progenitor cells collected by apheresis (HPC,A) from the mobilized donor will be initially assessed in the HAL and stored overnight at 4°C, if necessary. The product will be washed to remove platelets and adjusted to an appropriate cell concentration for incubation with Biotinylated CliniMACS TCRαβ reagent (biotin conjugated anti TCRαβ antibody) and CD19 reagent in the manufacturer provided media. The cells will be washed to remove unbound reagent before labeling with paramagnetic beads conjugated with anti-biotin antibody). These cells will be applied to the CliniMACS device and the depletion will be performed as described by the manufacturer.

After depletion is complete, the cells will be washed and resuspended in an infusion grade solution. The graft product will be enumerated and assessed for viable $CD34^+$ cell , α/β T-cell, and $CD19^+$ cell content by flow cytometry. The processed HPC,A product will be infused fresh or cryopreserved for future use after completion of release testing and evaluation. Cryopreservation will be performed per SOPs of the Human Applications Laboratory.

Target cell doses are listed in the following table:

Initial HPC Graft	Target Dose	Minimum Dose	Maximum Dose
$CD34^+$ cells/kg	≥ 5 x 10^6	2 x 10^6	50 x 10^6
$TCR\alpha\beta^+$ cells/kg	≤ 0.1 x 10^6	N/A	*0.1 x 10^6

* Maximum TCRαβ+ cell dose includes the sum of all TCRαβ-depleted products.

If the target CD34+ dose is not achieved in a single collection, up to two additional collections may be performed (see Section 4.4). These additional collections may be TCD on the CliniMACS device by TCRαβ-depletion, or by CD34+ cell enrichment (if the maximum TCRαβ+ cell dose has been reached). There is no target, minimum, or maximum cell dose for residual CD19+ cells.

4.6 Additional Progenitor Cell Graft Administration

Infusion of an additional HPC graft from the original or an alternative haploidentical donor may be performed for participants when clinically indicated for graft failure, poor immune reconstitution, or poor hematopoietic recovery. The use of and content of a conditioning regimen is left to the discretion of the PI and/or primary transplant attending such that the most appropriate therapy is chosen for the clinical situation. Participants who receive a conditioning regimen may remain on therapy but will not have further immune reconstitution and evaluation research labs collected.

The HPC graft will typically be obtained by apheresis (HPC,A) and be infused fresh. The target $CD34^+$ dose for this additional infusion is $\geq 5 \times 10^6$ cells/kg. If the participant has quiescent or active BOOP, BOS, acute Grade III-IV GVHD, or any other reason that a severely T-cell depleted graft may be indicated, then a graft from the donor processed on the CliniMACS™ device using either $CD34^+$ selection or CD3+ depletion methodology may be utilized.

4.7 Donor Lymphocyte Infusions

4.7.1 Prophylactic CD45RA-depleted DLI

This donor product will be processed for CD45RA+ depletion using the investigational CliniMACS device as directed by the manufacturer (Miltenyi Biotech). See section 5.2 for additional CliniMACS device information. Briefly, therapeutic cells collected by apheresis (MNC,A) from a non-mobilized donor will be initially assessed in the HAL and stored overnight at 4ºC, if necessary. The product will be washed to remove platelets and adjusted to an appropriate cell concentration for incubation with the CliniMACS CD45RA Microbead reagent in the manufacturer provided media. The cells will be washed to remove unbound microbeads. These cells will be applied to the CliniMACS device and the depletion will be performed as described by the manufacturer. The CD3+ cell target dosing will be determined by the protocol determined dose level (see section 2.4 or the table below). The goal CD45RA-depletion ≥2.0 log10 depletion of CD45RA+ cells, however a depletion t hat does not achieve this target level may still be released to the patient as long as th e maximum dose of CD3+CD45RA+ cells is no greater than 0.05×10^6/kg.

Dose level	CD3+ dose
0	1x10^5/kg + MMF through Day +60
1	1x10^5/kg
2	1x10^6/kg
3	1x10^7/kg

The donor non-moblized product may be collected anytime after donor consent is obtained, but care must be made to ensure patient is very likely to proceed with transplantation if the collection is performed prior to the patient initiating therapy. The collection may occur at any time prior to the initiation of G-CSF for mobilization, or any time that is at least 10 days after completion of mobilization. The DLI product may be collected as a whole blood unit donation or by leukapheresis (preferred). If the DLI is collected by standard phlebotomy, the volume to be collected would be approximately 300 ml whole blood. If the DLI is collected by leukapheresis, the amount to be processed would be approximately 2 total blood volumes.

Protocol prescribed CD45RA-depleted DLI for the original cohort was given no sooner than 2 weeks following donor neutrophil engraftment, and no later than Day +60. Patient had to be sufficiently recovered from mucositis to the point that they were tolerating PO feeds or approximately 50% of caloric needs by NG tube with absence of, or improving, diarrhea; and resolution of any narcotic need for abdominal pain. In addition, there must be no acute GVHD or it must be quiescent. ALT must be less than 5x ULN, bili less than or equal to 1.5x ULN, and creatinine less than or equal to 1.5x ULN.

For the **early DLI cohort** (Amendment 5), the goal is to give the primary prophylactic CD45RA-depleted DLI at the MED of dose level 3 (1x10^7/kg) around Day +14, with a goal range of Day +10 to +21. As above, any DLI given after Day +60 will not be considered prophylactic DLI per protocol and will have to be given based on clinical need (see Section 4.7.2.2). Unlike above, patients do not have to be recovered from mucositis. However, like above, there must be no acute GVHD or it must be quiescent. ALT must be less than 5x ULN, bili less than or equal to 1.5x ULN, and creatinine less than or equal to 1.5x ULN.

4.7.2 Additional DLI (based on clinical need)

Additional DLI may be given as needed as detailed below. It may be given with or without preceeding lymphodepleting chemotherapy. Recipients who are further out from transplant and with substantial lymphocyte engraftment may quickly tolerize the newly infused donor lymphocytes. In such patients, lymphodepleting chemotherapy may be needed for effective DLI activity. Regimen will vary based on patient condition, underlying disease, etc. Regimen will be determined by attending physician and/or PI, but in general would be low dose, expected to have minimal direct toxicity, and have limited or very transient effects on ANC.

Regimens for lymphodepleting chemotherapy will be defined in a separate document (non-protocol treatment plan; NPTP) and will require specific consent to the NPTP.

4.7.2.1 Conventional Donor Lymphocyte Infusions

DLI may be administered from the original donor for <100% donor chimerism, serious viral reactivation or infection, any evidence of disease/recurrence, or poor immune recovery (such as lymphopenia).

- Decreased donor chimerism is defined as any single chimerism test that is not 100% donor
- Serious viral reactivation is defined as any virus detected in the blood by PCR, with a 1 log increase in viral load despite antiviral medication.
- Serious viral infection is defined as any infectious disease from an identified virus that has progressed in severity despite antiviral therapy (if available) or shows no improvement despite 1 week of antiviral therapy (if available)
- Any evidence of disease/recurrence is defined as any flow cytometry, PCR, NGS (such as RNAseq), cytogenetic, or any other validated molecular testing that detects the presence of the original hematologic malignancy within the limits of that particular test.

The DLI collected may be collected as a whole blood unit donation or by leukapheresis. If the DLI is collected by standard phlebotomy, the volume to be collected would be 300 - 500 ml whole blood. If the DLI is collected by leukapheresis, the amount to be processed would be approximately 2 total blood volumes.

Prior to administration of DLI, any active immunosuppression should be withdrawn and the recipient should have no active GVHD. The initial dose will typically be 2.5 or 5 x 10^4 $CD3^+$/kg. Subsequent doses will be administered at approximately 2 to 4-week intervals with escalating doses of T cells if no moderate or severe GVHD occurs with the prior DLIs. The typical initial dose escalation for patients on this protocol is presented in the following table:

CONVENTIONAL DLI DOSE AND SCHEDULE		
DLI	**Dose(10^4 $CD3^+$/kg)**	**Comments**
Initial Dose	2.5	Approximately 2-4 week interval If no moderate or severe GVHD
Dose #2	5	
Dose #3	10	

Although this algorithm will be appropriate for a majority, the treating transplant attending physician may alter the dose and/or interval of DLI based on response to previous DLI, the severity of the clinical situation, and the condition of the patient. There is a low threshold for providing an initial dose of 2.5 x 10^4 $CD3^+$/kg. DLI at this dose level have not been associated with GVHD. Therefore, DLI may be initiated for any chimerism less than 100% in a patient who is off immune suppression. The risk of GVHD increases with increasing doses.

4.7.2.2 CD45RA-depleted Donor Lymphocyte Infusions (additional)

Low dose conventional DLI has been found to be very effective for treatment of low level mixed chimerism, but it's efficacy in serious viral infections and recurrent disease (even at MRD levels) is poor. Although prophylactic memory T-cell DLI is given as a part of this transplant, some patients may have recurrence or persistence of lymphopenia due to infections or post-transplant immunosuppressive drugs (such as corticosteroids). Therefore selected patients may benefit from additional adoptive transfer of donor memory cell populations in the post-transplant setting. This would include: 1. Patients with viral reactivation or infections that are not responding to antiviral therapy or historically have a poor response to antiviral therapy and cannot receive viral specific CTL due to ineligibility or unavailability; 2. Patients with persistant <100% donor chimerism despite low dose conventional DLI; 3. Patients who have evidence of disease/recurrence, particularly molecular or MRD levels of relapse; 4. Patients who have poor immune recovery (such as lymphopenia), defined as ALC <500/uL and/or TCRαβ T-cell count <300/uL. To receive additional CD45RA-depleted DLI, patients must meet at least one of the above criteria and be at least 4 weeks following preemtive DLI. They should preferrably be off immune suppression and must have no evidence of active GVHD (any prior GVHD must be quiescent). These patients will be allowed to receive CD45RA-depleted DLI in the following doses:

CD45RA-DEPLETED DLI DOSE AND SCHEDULE		
DLI	**Dose(10^6 $CD3^+$/kg)**	**Comments**
Initial Dose	0.1	Approximately 2-4 week interval If no moderate or severe GVHD
Dose #2	1	
Dose #3	10	

If the prophylactic DLI failed to achieve the goal memory cell reconstitution, the initial dose for this additional CD45RA-depleted DLI may be started at the next dose level up from the prophylactic dose provided.

The DLI may be collected as a whole blood unit donation or by leukapheresis. If the DLI is collected by standard phlebotomy, the volume to be collected would be approximately 300 ml whole blood. If the DLI is collected by leukapheresis, the amount to be processed would be approximately 2 total blood volumes. The collected donor lymphocytes would then undergo CD45RA depletion as decribed above (section 4.7.1).

The release criteria for the CD45RA-depleted DLI will include a maximum dose of CD3+CD45RA+ cells no greater than $0.05x10^6$/kg.

Conventional DLI and CD45RA-depleted DLI cannot be given within 14 days of each other.

4.8 Quality Assurance of Cellular Products

Quality assurance for cell products is overseen by the TPQ Quality Assurance division, which authorizes release of all products. Only trained cell processors will process the cell

products. A labeling and product tracking system is in place to ensure that the correct cells are infused into the research participant.

Assays of cell numbers and immunophenotyping will be performed both before cell processing and at critical stages of the process. These values will be recorded according to SOP of the HAL. All products will be tested for viability and sterility (culture and Gram stain). Culture results are not available before infusion of cell products. If the gram stain is positive, the research participant/parent and/or guardian will be informed of this event and of the risks of proceeding prior to infusion. Positive results will be investigated as per the variance procedures of the HAL. The IRB and FDA will be notified, if at any time after infusion, cell product was determined to be contaminated.

5.0 DRUG/DEVICE/BIOLOGIC AGENT INFORMATION

5.1 Medications

Anti-thymocyte globulin (rabbit) (Thymoglobulin®, rabbit ATG)	
Source & Pharmacology	Anti-thymocyte globulin is a purified, pasteurized, gamma immune globulin, obtained by immunization of rabbits with human thymocytes. This immunosuppressive product contains cytotoxic antibodies directed against antigens expressed on human T-lymphocytes. The mechanism of action by which polyclonal antilymphocyte preparations suppress immune responses is not fully understood. Possible mechanisms by which anti-thymocyte globulin may induce immunosuppression in vivo include: T-cell clearance from the circulation and modulation of T-cell activation, homing and cytotoxic activities. Anti-thymocyte globulin (rabbit) includes antibodies against T-cell markers such as CD2, CD3, CD4, CD8, CD11a, CD18, CD25, CD44, CD45, HLA-DR, HLA Class 1 heavy chains, and 2 microglobulin. T-cell depletion is usually observed within a day from initiating anti-thymocyte globulin therapy.
Formulation and Stability	Anti-thymocyte globulin (refrigerated) is available as sterile, lyophilized powder to be reconstituted with sterile diluent (both lyophilized powder and diluent should be at room temperature before reconstitution). Reconstituted solutions provide a final concentration of 5mg/ml x 5ml. The product must be further diluted in normal saline prior to administration. Infusion solutions may be administered over 2 to 6 hours depending on dose and should be prepared immediately prior to administration.
Supplier	Commercially available
Toxicities	Frequently reported evens include fever, chills, leukopenia, pain, headache, abdominal pain, diarrhea, hypertension, nausea, thrombocytopenia, peripheral edema, dyspnea, asthenia, hyperkalemia, tachycardia. The most serious toxicity is that of anaphylaxis. Supportive medical resources must be readily available for patient management anaphylaxis precludes further administration of the drug. The dose must be administered over at least 4 hours and the patient pretreated with antihistamine, corticosteroid, and antipyretic.
Route	Intravenous

Blinatumomab (Blincyto)	
Source & Pharmacology	Blinatumomab is a bispecific CD19-directed CD3 T-cell engager indicated for the treatment of Philadelphia chromosome-negative relapsed or refractory B-cell precursor acute lymphoblastic leukemia. It activated endogenous T cells by connecting CD3 in the T-cell receptor complex with CD19 on benign and malignant B cells. Blinatumomab mediates the formation of a synapse between the T-cell and the tumor cell, upregulation of cell adhesion molecules, production of cytolytic proteins, release of inflammatory cytokines, and proliferation of T cells, which result in redirected lysis of CD19+ cells. During the continuous intravenous infusion over 4 weeks, the phamacodynamic response was characterized by T-cell activation and initial redistribution, reduction in peripheral B cells, and transient cytokine elevation. The pharmacokinetics of blinatumomab appear linear over a dose range from 5 to 90 mcg/m2/day in adult patients. Following continuous intravenous infusion, the steady-state serum concentration was achieved within a day and remained stable over time.
Formulation and Stability	Blinatumomab is available as 35mcg of lyophilized powder in a single-use vial for reconstitution.
Supplier	Commercially available
Toxicities	The most common adverse reactions (≥20%) were pyrexia, headache, perhipheral edema, febrile neutropenia, nausea, hypokalemia, tremor, rash, and constipation. Cytokine Release Syndrome and Neurologic toxicities that are severe, which can be life-threatening or fatal, have occurred with blinatumomab and necessitate interruption or discontinueation of blinatumomab.
Route	Continuous intravenous infusion

Cyclophosphamide (Cytoxan)	
Source & Pharmacology	Cyclophosphamide is a nitrogen mustard derivative. It acts as an alkylating agent that causes cross-linking of DNA strands by binding with nucleic acids and other intracellular structures, thus interfering with the normal function of DNA. It is cell cycle, phase non-specific. Cyclophosphamide is well absorbed from the GI tract with a bioavailability of >75%. It is a prodrug that requires activation. It is metabolized by mixed function oxidases in the liver to 4-hydroxycyclo-phosphamide, which is in equilibrium with aldophosfamide. Aldofosfamide spontaneously splits into nitrogen mustard, which is considered to be the major active metabolite, and acrolein. In addition, 4-hydroxycy-clophosphamide may be enzymatically metabolized to 4-ketocyclophosphamide and aldophosfamide may be enzymatically metabolized to carboxyphosphamide that is generally considered inactive. Cyclophosphamide and its metabolites are excreted mainly in the urine.

	Dose adjustments should be made in patients with a creatinine clearance of <50 ml/min.
Formulation and Stability	Cyclophosphamide is available in vials containing 100, 200, 500, 1000 and 2000mg of lyophilized drug and 75 mg mannitol per 100 mg of cyclophosphamide. Both forms of the drug can be stored at room temperature. The vials are reconstituted with 5, 10, 25, 50 or 100 ml of sterile water for injection, respectively, to yield a final concentration of 20 mg/ml. Reconstituted solutions may be further diluted in either 5% dextrose or 0.9% NaCl containing solutions. Diluted solutions are physically stable for 24 hours at room temperature and 6 days if refrigerated, but contain no preservative, so it is recommended that they be used within 24 hours of preparation.
Supplier	Commercially available
Toxicities	Dose limiting toxicities of cyclophosphamide includes BM suppression and cardiac toxicity. Cardiac toxicity is typically manifested as congestive heart failure, cardiac necrosis or hemorrhagic myocarditis and can be fatal. Hemorrhagic cystitis may occur and necessitates withholding therapy. The incidence of hemorrhagic cystitis is related to cyclophosphamide dose and duration of therapy. Forced fluid intake and/or the administration of mesna decreases the incidence and severity of hemorrhagic cystitis. Other toxicities reported commonly include nausea and vomiting (may be mild to severe depending on dosage), diarrhea, anorexia, alopecia, immunosuppression and sterility. Pulmonary fibrosis, SIADH, anaphylaxis and secondary neoplasms have been reported rarely.
Route	Intravenous infusion

Fludarabine (Fludara)	
Source & Pharmacology	Fludarabine phosphate is a synthetic purine nucleoside analog. It acts by inhibiting DNA polymerase, ribonucleotide reductase and DNA primase by competing with the physiologic substrate, deoxyadenosine triphosphate, resulting in inhibition of DNA synthesis. In addition, fludarabine can be incorporated into growing DNA chains as a false base, thus interfering with chain elongation and halting DNA synthesis. Fludarabine is rapidly dephosphorylated in the blood and transported intracellularly via a carrier-mediated process. It is then phosphorylated intracellularly by deoxycytidine kinase to the active triphosphate form. Approximately 23% of the dose is excreted as the active metabolite in the urine (with dosages of 18-25 mg/m^2/day for 5 days). Renal clearance appears to become more important at higher doses, with approximately 41-60% of the dose being excreted as the active metabolite in the urine with dosages of 80-260 mg/m^2.

Formulation and Stability	Fludarabine is supplied in single-dose vials containing 50 mg fludarabine as a white lyophilized powder and 50 mg of mannitol. The intact vials should be stored under refrigeration. Each vial can be reconstituted by adding 2 ml of sterile water for injection resulting in a final concentration of 25 mg/ml. Because the reconstituted solution contains no antimicrobial preservative, the manufacturer recommends that it should be used within 8 hours of preparation. The solution should be further diluted in 5% dextrose or 0.9% NaCl prior to administration.
Supplier	Commercially available.
Toxicities	The major dose-limiting toxicity of fludarabine is myelosuppression. Nausea and vomiting are usually mild. Side effects reported commonly include anorexia, fever and chills, alopecia and rash. Neurotoxicity can be manifested by somnolence, fatigue, peripheral neuropathy, mental status changes, cortical blindness and coma and is more common at high doses. Neurotoxicity is usually delayed, occurring 21-60 days after the completion of a course of therapy and may be irreversible. Side effects reported less commonly include diarrhea, stomatitis, increased liver function tests, liver failure, chest pain, arrhythmias and seizures. Pulmonary toxicity includes allergic pneumonitis characterized by cough, dyspnea, hypoxia and pulmonary infiltrates. Drug induced pneumonitis is a delayed effect, occurring 3-28 days after the administration of the third or later course of therapy. Administration of corticosteroids usually results in resolution of these symptoms.
Route	Intravenous

G-CSF (Filgrastim, NeupogenÒ)	
Source & Pharmacology	G-CSF (granulocytic colony stimulating factor), is a biosynthetic hematopoietic agent that is made using recombinant DNA technology in cultures of Escherichia coli. G-CSF stimulates production, maturation and activation of neutrophils. In addition, endogenous G-CSF enhances certain functions of mature neutrophils, including phagocytosis, chemotaxis and antibody--dependent cellular cytotoxicity.
Formulation and Stability	G-CSF is supplied in vials containing 300 mcg and 480 mcg of G-CSF at a concentration of 300mcg/ml. The intact vials should be stored under refrigeration. The vials can be left out of refrigeration for 24 hours, but should be discarded if left at room temperature for longer periods of time. G-CSF can be drawn up into tuberculin syringes for administration and stored under refrigeration for up to 7 days prior to usage. G-CSF can be further diluted for intravenous infusion in 5% dextrose. Do not dilute in saline---precipitate may form. If the final concentration of this product is <15 mcg/ml, it is recommended that albumin be added to a final concentration of 2mg/ml (0.2%) to minimize adsorption of the drug to infusion containers and equipment.
Supplier	Commercially available.
Toxicities	G-CSF causes marked leukocytosis. Adverse reactions reported commonly

	include bone pain, thrombocytopenia, diarrhea, nausea, rash, alopecia, fever, anorexia and pain or bruising at the injection site. Allergic reactions, MI, atrial fibrillation, and splenomegaly have been reported rarely. G-CSF is contraindicated in research participants with allergy to *E. coli* derived products.
Route	Intravenous or subcutaneous.

Melphalan (L-phenylalanine mustard, phenylalanine mustard, L-PAM, L-sarcolysin, Alkeran[â])	
Source & Pharmacology	Melphalan, a derivative of nitrogen mustard, is a bifunctional alkylating agent. Its chemical name is 4-[bis(2-chloroethyl)amino]-L-phenylalanine, and it has a molecular weight of 305.20. Melphalan is active against tumor cells that are actively dividing or at rest. Its cytotoxicity is thought to be due to inter-strand cross-linking with DNA, probably by binding at the N7 position of guanine. Melphalan is highly protein bound and does not penetrate well into the cerebral spinal fluid. Elimination half-life after intravenous administration in adults is approximately 75 minutes. Elimination appears to be primarily by chemical hydrolysis, but caution should be used in patients with renal impairment. Plasma concentrations of melphalan after oral administration are highly variable, possibly due to incomplete absorption, variable "first pass" hepatic metabolism or rapid hydrolysis. Area under the plasma concentration-time curves for orally administered melphalan is approximately 60% of intravenously administered melphalan in adult studies.
Formulation and Stability	Available as 2 mg tablets for oral administration. This medication is stable at room temperature until expiration date on the packaging. Intravenous formulation is supplied as 50 mg freeze dried glass vial. Each 50 mg vial is supplied in a carton containing a 10 ml vial of sterile diluent. Lyophilized melphalan should be stored at controlled room temperature and protected from light. Each vial is marked with its expiration date. The melphalan for injection must be reconstituted immediately prior to infusion by rapidly adding the contents of the diluent vial (10 ml) to the freeze dried powder with a 20 gauge or larger sterile needle and immediately shaking vigorously until a clear solution is obtained. This results in a 5 mg/ml solution. The dose should then be diluted in 0.9% NaCl for injection to a final concentration of not greater than 0.45 mg/ml. The resulting admixture should be infused over a minimum of 15 minutes. The infusion should be completed within 60 minutes of reconstitution. Do not refrigerate the reconstituted melphalan.
Supplier	Commercially available
Toxicities	Melphalan is cytotoxic and caution should be used in handling and preparing the solution or administering the tablets. Use of gloves is recommended, and if contact with skin or mucosa occurs, immediately wash thoroughly. Second cancers such as acute non-lymphocytic leukemia, myeloproliferative syndrome, and carcinoma have been reported in patients taking melphalan alone or in combination with other chemotherapy or

	radiation. Melphalan causes suppression of ovarian function in premenopausal women, with a significant number of patients having amenorrhea. Testicular suppression (reversible and irreversible) has been reported. The most common adverse reaction is myelosuppression. Irreversible BM failure has been reported. Gastrointestinal side effects reported include nausea/vomiting, diarrhea and oral mucosa ulceration. Hepatic toxicity has occurred, including veno-occlusive disease. Acute hypersensitivity reactions occur in about 2.4% of patients, and can include anaphylaxis. Hypersensitivity reactions were characterized by urticaria, pruritus, and edema. Some patients exhibited tachycardia, bronchospasm, dyspnea and hypotension that responded to antihistamines and corticosteroids. Other side effects that have been reported include skin ulceration or necrosis at injection site, vasculitis, alopecia, hemolytic anemia, pulmonary fibrosis, and interstitial pneumonitis.
Route	Intravenous

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

Thiotepa (ThioplexÒ by Immunex) (TESPA, TSPA)	
Source & Pharmacology	Thiotepa is a cell-cycle nonspecific polyfunctional alkylating agent. It reacts with DNA phosphate groups to produce cross-linking of DNA

	strands leading to inhibition of DNA, RNA and protein synthesis. Thiotepa is extensively metabolized in the liver to metabolites that retain activity, primarily triethylene-phosphoramide (TEPA). The main route of elimination is via the urine, mainly as metabolites; the elimination half-life of the thiotepa is 2.5 hours, and that of TEPA is 17.6 hours.
Formulation and Stability	Thiotepa is supplied in single-use vials containing 15 mg of lyophilized thiotepa, 80 mg NaCl and 50 mg NaHCO3. The intact vials should be stored under refrigeration and protected from light. Each vial should be reconstituted with 1.5 ml of sterile water for injection to yield a concentration of 10 mg/ml. Further dilution with sterile water for injection to a concentration of 1 mg/ml yields an isotonic solution; if larger volumes are desired for intracavitary, intravenous infusion, or perfusion therapy, this solution may then be diluted with 5% dextrose or 0.9% NaCl containing solutions. The 10 mg/ml reconstituted solution is chemically stable when stored in the refrigerator for up to 5 days, however, it is recommended that solutions be prepared just prior to administration since they do not contain a preservative. Reconstituted solutions should be clear to slightly opaque: the solutions may be filtered through a 0.22 micron filter to eliminate haze.
Supplier	Commercially available; manufactured by Immunex
Toxicities	Dose limiting toxicity is myelosuppression. The leukocyte nadir may occur at any time from 10 to 30 days. Other toxicities include pain at the injection site, nausea and vomiting, anorexia, mucositis, dizziness, headache, amenorrhea, interference with spermatogenesis, and depigmentation with topical use. Allergic reactions, including skin rash and hives, have been reported rarely. Rare cases of apnea, hemorrhagic cystitis, and renal failure have occurred. Thiotepa is mutagenic, carcinogenic, and teratogenic in animals. Pregnancy category D.
Route	Intravenous infusion.

5.2 CliniMACS^{TM} System

The mechanism of action of the CliniMACS Cell Selection System is based on magnetic-activated cell sorting (MACS). The CliniMACS device is a powerful tool for the isolation of many cell types from heterogeneous cell mixtures, (e.g. apheresis products). These can then be separated in a magnetic field using an immunomagnetic label specific for the cell type of interest, such as $CD3^+$ human T cells.

The cells to be isolated are specifically labeled with super-paramagnetic particles by an anti-body directed toward a cell surface antigen. After magnetic labeling, the cells are separated using a high-gradient magnetic separation column as described below. The magnetically labeled cells are retained in the magnetized column while the unlabeled cells flow through the column for collection. The retained cells are eluted by removing the magnetic field from the column, washing the cells out and collecting them in a separate container from the unlabeled cells.

The super-paramagnetic particles are small in size (about 50 nm in diameter) and are composed of iron oxide/hydroxide and dextran conjugated to monoclonal antibodies. These magnetic particles form a stable colloidal solution and do not precipitate

or aggregate in magnetic fields. The antibody conjugated beads used in this system are highly specific (e.g. CD3+ conjugated beads). High-gradient MACS technology has been shown to achieve rapid and highly specific depletion or enrichments of large numbers of target cells from BM, cord blood, and normal peripheral blood mononuclear cells.

The CliniMACS device incorporates a strong permanent magnet and a separation column with a ferromagnetic matrix to separate the cells labeled with the magnetic particles. The high-gradient system allows the application of strong magnetic forces and a rapid de-magnetization. Small ferromagnetic structures, such as the column matrix, placed in a magnetic field concentrate this homogenous field and thereby produce high magnetic gradients. In their immediate proximity, the ferromagnetic structures generate magnetic forces 10,000-fold greater than in the absence of those structures enabling the retention of magnetically labeled cells. After removing the column from the magnet, the rapid de-magnetization of the column matrix allows the release of retained cells.

The CliniMACS device is comprised of a computer controlled instrument incorporating a strong permanent magnet, a closed-system sterile tubing set containing columns with a coated ferromagnetic matrix and a paramagnetic, cell specific, labeling reagent. The instrument will separate the cells in a fully automated process yielding a cell population highly depleted of a specific cell population. The CliniMACS device is not licensed by the FDA for TCRαβ depletion or CD45RA depletion and therefore is investigational.

The CliniMACS device has separate programs that allow cell selection procedures optimized for either depletion (e.g. $CD3^+$, $TCR\alpha\beta^+$, $CD45RA^+$) or selection of a target cell population (e.g. $CD34^+$ or $CD56^+$ cells). The basic mechanism is the same for either application; target cells are "tagged" with super-paramagnetic particles and eventually separated from the unlabeled cells using the CliniMACS device as described above. The desired target cells can either be infused or discarded appropriately.

6.0 REQUIRED EVALUATIONS, TESTS, AND OBSERVATIONS

6.1 Schedule of Evaluations

All evaluations for these participants will be carried out as outlined in Appendix D, and guided by the Standard Operating Procedures (SOPs) of the St. Jude Children's Research Hospital, Department of BMTCT, for recipients of allogeneic stem cell transplantation. Copies of these SOPs and ongoing updates can be found at the following site: Please note that all relevant SOPs can be found on the BMT&CT Clinical Transplant Program intranet page.

Furthermore, to accommodate the research studies, flexibility in the date is allowed without a deviation from protocol. The degree of flexibility in the timing is also provided in Appendix D.

6.2 Long-term Follow-up Evaluations

In general, recipients of allogeneic HCT at St. Jude are seen at least annually until 10 years post-transplant in the Department of BMTCT outpatient clinic or in the after completion

of therapy (ACT) clinic. For the purpose of this study, research participants will be followed to year 1 post-transplantation.

6.3 Evaluation for Chimerism and Engraftment

Evaluation for chimerism and engraftment will be performed on bone marrow or peripheral blood samples according to the timelines noted in Appendix D. Bone marrow chimerism studies will be conducted on or about the following time points: day +21, day +100, and year one post-transplant. However, for research participants who have less than 100% donor chimerism at or about day +21 post-transplant, a repeat bone marrow study (to include chimerism) may be performed as indicated.

The time to neutrophil and platelet engraftment will be recorded. Neutrophil engraftment will be defined as the first of 3 consecutive days of an absolute neutrophil count (ANC) greater than or equal to 500/mm^3. Time to platelet engraftment will be designated as the time to platelet count exceeding 20,000/mm^3 and 50,000/mm^3 without a platelet transfusion in the preceding seven days.

Chimerism studies using peripheral blood will be obtained on an approximate weekly basis according to the evaluation schedule noted in Appendix D. Additional bone marrow and/or peripheral blood chimerism studies may be performed throughout the course of this study when clinically indicated. Chimerism studies derived from bone marrow may be used in lieu of a specified peripheral blood sample if a bone marrow sample is available. In the event of graft failure/rejection, subsequent chimerism studies may be held as they would not be clinically indicated at that time.

If there is an initial decrease in donor chimerism to less than 90% at any time on peripheral blood studies, a bone marrow examination will subsequently be performed within approximately two weeks to confirm this initial decline. In addition, chimerism analysis may be performed in subsets of lymphocytes, granulocytes, and monocytes for research participants with increasing host chimerism until the research participant attains 100% donor chimerism. Chimerism studies will be performed in the St. Jude Department of Pathology using standard techniques. Chimerism studies will be reported in the database as donor percentages.

6.4 Evaluation for Immune Reconstitution

Performed according to the schedule outlined in Appendix D until the immune parameters recover to normal level or donor pattern:

6.4.1 Lymphocyte subsets study: Flow cytometry enumeration.

6.4.2 Quantitative immunoglobulins: IgG, IgM, and IgA levels.

6.4.3 VBETA/TREC Research: Thymic output and T cell repertoire.

6.4.4 Lymphocyte Research: Lymphocyte subset number and function.

6.4.5 Phenotype Research: Phenotype number and function.

6.4.6 IR-Phenotype: Immune reconstitution of memory and naïve T cells will be investigated in depth in the Youngblood Lab. This may include phenotypic subset

characterization, functional correlates, and analysis of the epigenetic signatures of these populations.

6.4.7 T-Function: T-cell function will be investigated in depth in the Thomas Lab. This may include antigen-specific T-lymphocyte response to viral infections, such as herpes viruses (CMV, HSV and VZV) and respiratory viruses (influenza and RSV).

6.5 Evaluations for ATG pharmacokinetics testing (optional)

PK testing of rabbit ATG will be obtained in HCT recipients to evaluate drug exposure and to determine the length of time that ATG remains active following HCT. 2mL of peripheral blood will be drawn into a heparinized (dark green top) tube at the following timepoints for the original cohort: pre- and post- 3rd dose of ATG on Day -3, then once each on Day -1, 0, +2, +4, +7, +14, and +21 (total samples = 9). Sample collection date may be adjusted ±2 days as needed (such as to accommodate blood volume concerns, line access issues, etc.). The ±2 days adjustment will not apply to the Day -3, -1, 0, +2 and +4 samples.

For the **early DLI cohort**, samples will be obtained pre- and post- 3rd dose of ATG on Day -10, then once each on Day -8, -7, -5, -3, 0, +7, and +14 (total samples = 9). The ±2 days adjustment will not apply to the Day -10, -7, -5, and -3 samples.

6.6 Minimal Residual Disease Evaluation

Minimal residual disease (MRD) assays in BM by immunologic and molecular methods[54-55] will be performed for those research participants who have had this test performed during prior therapy for their disease at St. Jude only or those for who samples of diseased marrow were available to identify a leukemic marker for MRD testing. MRD assays may be performed more frequently in participants with increasing host chimerism. Tests will be performed in the appropriate St. Jude laboratories.

6.7 Research Tests on Haploidentical Donor (optional)

Donors will be offered the option for participation in research studies of immune reconstitution. These tests will be obtained after consent and preferably prior to growth factor administration. Lymphocyte subset analysis of the donor appears to allow for the prediction of the reconstitution of the lymphocyte subsets in the research participant after transplantation. Data in larger donor/research participant pairs will help to verify these observations. A list of these optional research studies are noted in Appendix D and detailed below:

6.7.1 Lymphocyte subsets study: Flow cytometry enumeration.

6.7.2 VBETA/TREC Research: Thymic output and T cell repertoire.

6.7.3 Lymphocyte Research: Lymphocyte subset number and function.

6.7.4 Phenotype Research: Phenotype number and function.

6.7.5 IR-PHENOTYPE, and T-FUNCTION: In depth characterization of donor lymphocytes

7.0 EVALUATION CRITERIA

7.1Toxicity Evaluation Criteria

Adverse event (AE) monitoring for on-study research participants will be assessed using the NCI Common terminology Criteria for Adverse Events Version 5.0. The standard procedures for adverse event collection and monitoring are noted in Appendix C. An exception will be made with the staging and grading of acute and chronic GVHD, which will be assessed by the criteria defined in Appendix B.

7.2 Hematologic Recovery Criteria

Post-transplant hematologic recovery will be determined using the engraftment criteria as follows:

7.2.1Neutrophil engraftment will be defined as the first of 3 consecutive tests performed on different days of an ANC $\geq 500/mm^3$ with evidence of donor cell engraftment.

7.2.2Platelet engraftment will be defined as the first of 3 consecutive tests performed on different days of a platelet count $\geq 20{,}000/mm^3$ with no platelet transfusions in the preceding 7 days.

7.3 Graft Failure Criteria

7.3.1 Primary graft failure will be defined as an ANC never meeting or exceeding $500/mm^3$ for 3 consecutive tests performed on different days and no evidence of donor chimerism (<5%) by day +30 post-HCT.

7.3.2 Secondary graft failure will be defined as a decline in ANC to$<500/mm^3$ with a decline in donor chimerism to <5% in research participants with prior engraftment.

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

8.1 Recipient Criteria

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

8.1.1 Withdrawal from protocol.

8.1.2 Death.

8.1.3 Donor unable to provide the HPC required for intended recipient to undergo the primary HCT procedure (refer to donor off-study criteria).

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

8.1.5 One year after HPC infusion (i.e. has completed the year +1 post-primary transplant evaluation).

8.1.6 Development of a significant change in health status at any point of therapy which would render receipt of the transplantation procedure or continuation in the study medically unsafe or not in the participant's best interest.

8.2 Off-therapy Criteria

Recipient research participants may remain on study, but considered off therapy, for monitoring if one of the following occurs (off therapy participants monitored for disease status and survival only):

8.2.1 Requires conventional chemotherapy for confirmed (generally >5%) disease relapse.

Epigenetic or targeted therapy, immune therapy (such as repeat dosing of blinatumomab, or use of checkpoint inhibitor), and low dose lymphodepleting chemotherapy with DLI, even if molecularly detectible disease is present, would not trigger this off-therapy criteria.

8.2.2 Experiences graft failure/rejection, requiring non-protocol therapy other than as described in Section 4.6 of this protocol.

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

8.2.4 Positive pregnancy test post-HPC infusion.

8.2.5 Recipient requires an additional HPC infusion, and unable to receive these cells due to donor issues.

8.3 Donor Criteria

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

8.3.1 Withdrawal from protocol. Donor research participants may withdraw their consent to participate at any time. Physician may withdraw donor at any time that continuation in the study is deemed medically unsafe or no longer in the donor's best interest.

8.3.2 Day of transplant recipient or transplant donor death, whichever occurs first.

8.3.3 Development of a change in health status, including positive pregnancy test or a clinically significant risk for or positive testing for communicable disease, which in the opinion of the PI, would render the donor medically ineligible to serve (or continue to serve) as a therapeutic cell donor.

8.3.4 Unable to be contacted and/or effectively monitored by PI and/or designees for follow-up as judged by the PI (including non-compliance and lost to follow-up).

8.3.5 Once the PI has determined that the transplant recipient does/will not require an additional infusion(s) of the donor's cells for the purpose of this protocol, the donor will be taken off study seven days post final cell collection procedure.

9.0 SAFETY AND ADVERSE EVENT REPORTING REQUIREMENTS

9.1 Reporting Adverse Experiences and Deaths to St. Jude IRB

The principal investigator is responsible for promptly reporting to the IRB any adverse events that are unexpected/unanticipated, serious, and that may represent potential harm or increased risk to research participants. When an unexpected death occurs, the PI should report it to the Director of Human Subject's Protection immediately. A reportable event submission should follow within 48 hours of notification of the event.

Serious, unexpected, and related or possibly related events must be reported within 10 business days of notification of the event. At the same time, the investigator will notify the study sponsor and/or the FDA, as appropriate. All other SAEs, including expected death, and all captured AEs will be reported to the IRB at the time of the continuing reviews, with the following exception:

- Any grade III-IV infusion reactions will be reported as soon as possible but every effort should be made to assure reporting is no more than within 10 business days of the event.

For this research study, recipient participants will be followed for all NCI Grade III-V adverse events from the start of conditioning and throughout the first year post HCT, regardless of their relationship to the treatment given. In addition, clinically significant NCI Grade I-II adverse events that are judged to be related/possibly related may be collected per the discretion and judgment of the PI. Examples of "clinically significant Grade I-II adverse events could include, but are not limited to: events meeting criteria for SAE, infections requiring oral systemic therapy, VOD or hemorrhagic cystitis. GVHD events will be recorded on an ongoing basis regardless of stage or grade using the criteria defined in Appendix Band will not be graded according to NCI criteria.

With regard to the haploidentical donor participants, they will be followed for all SAEs and any clinically significant AEs, per the judgment of the PI, that are deemed related to the mobilization and/or apheresis procedure from the time of the initiation of mobilization growth factors, until 7-days post last day of the final apheresis procedure. If the transplant recipient requires a second HPC infusion, meaning that the donor is required to undergo the mobilization and apheresis procedure again, collection of this donor safety data will restart upon the initiation of the subsequent mobilization procedure and continue until 7-days post the last day of this apheresis procedure. Timelines for reporting of these donor events to the institutional and federal governing agencies will be according to the same timelines utilized for the recipients. A listing of the captured donor safety data will be provided in a separate table from the transplant recipients within each respective continuing review report.

The following definitions apply with respect to reporting adverse experiences:

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

- results in death;
- is life-threatening (places the participant at immediate risk of death from the event as it occurred);
- requires inpatient hospitalization or prolongation of existing hospitalization
- results in a persistent or significant disability/incapacity;
- results in a congenital anomaly/birth defect; or

- any other adverse event that, based upon appropriate medical judgment, may jeopardize the participant's health and may require medical or surgical intervention to prevent one of the above outcomes.

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

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

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

9.2 Reporting to St. Jude Institutional Biosafety Committee

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

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

The FDA will be notified in writing (IDE safety report) of any serious and unexpected AE associated with an investigational treatment or device; or any results from laboratory animal tests that suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Each notification to the FDA should be made as soon as possible and no later than 15 calendar days after the sponsor's initial receipt of the information. The FDA may require additional data to be submitted. In each written IND safety report, the sponsor shall identify all safety reports previously filed with the IND concerning a similar adverse experience, and shall analyze the significance of the adverse experience in light of the previous, similar reports where applicable.

The sponsor shall also notify the FDA by telephone, email, or by facsimile transmission of any unexpected fatal or life-threatening experience associated with the use of the investigational device as soon as possible but no later than 7 calendar days after the sponsor's initial receipt of the information. Any grade III-IV infusion reactions will be reported as soon as possible but every effort should be made to assure reporting is no more than within 7 business days of the event. Follow-up information to a safety report must be submitted as soon as the relevant information is available.

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

9.4 Reporting to St. Jude Office of Regulatory Affairs

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

9.5 Continuing Review Reports

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

9.6 Reporting to Miltenyi Biotec

Data related to device malfunction will be reported to Miltenyi Biotec, the manufacturer of the CliniMACS system. These device related malfunctions as well as any device related adverse events will be reported so the manufacturer can collect data and address those issues appropriately.

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

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

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

Data resulting from this transplant procedure will be sent for general registry purposes to comply with the federal government requirements. This information for both donor and recipient is submitted using a unique participant identification number. The information submitted for haploidentical recipients is less extensive than recipients of other donor products. For this reason, variables submitted may include but are not limited to the transplant recipient's date of birth, country/state of current residence, diagnosis, basic lympho-hematopoietic reconstitution (e.g. date of ANC and platelet engraftment), post-HCT disease status, and basic AEs (e.g. GVHD- yes or no), survival status, date/cause of death.

9.8 Data and Safety Monitoring Board

This study has been referred to the St. Jude Data and Safety Monitoring Board (DSMB) for regular monitoring, and will be sent to the DSMB upon approval by the St. Jude CT-SRC and IRB. The DSMB is charged with advising the Director and other senior leaders of St. Jude Children's Research Hospital (SJCRH) on the safety of clinical protocols being conducted by SJCRH investigators and on their continuing scientific validity.

10.0 DATA COLLECTION, STUDY MONITORING, AND CONFIDENTIALITY

10.1 Data Collection and Submission

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

10.2 Study Monitoring

This study will be monitored according to a study specific clinical trial monitoring plan (CTMP). The CTMP is based on the current St. Jude Children's Research Hospital Institutional Plan for Data and Safety Monitoring of Clinical Trials (DSMP) which outlines the monitoring strategy based on the risk of the study with an emphasis on participant safety, data quality, and human subjects protections. Additionally, the CTMP

is a supplement to the protocol, Clinical Trial Operations SOPs and study specific materials which will aid in the monitoring of this study.

The Investigator will permit study-related monitoring by providing direct access to source data and to the participants' medical histories. The Investigator will also maintain an Investigator Site File with essential documents according to ICH and all applicable regulatory guidelines and make the file accessible for monitoring visits as needed.

The Monitor will visit the study site at regular intervals during the study. Visits may be performed onsite, remotely, or in a hybrid fashion (both onsite and remote) as needed for the sponsor (SJCRH) to fulfill their obligations. Site monitoring is conducted according to applicable ICH and GCP guidelines to ensure the protection of participants' rights and well-being, protocol adherence, quality of data (accurate, complete, and verifiable), study treatment accountability, compliance with regulatory requirements, and continued adequacy of the investigational site and its facilities.

10.3 Confidentiality

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

10.4 Genomic Data Sharing

Genomic and epigenomic data may be shared through the St. Jude Cloud, the Database for Genotypes and Phenotypes (dbGAP) and the Gene Expression Omnibus (GEO), which are both run by the NIH, and the Sequence Read Archive (SRA). Prior to submitting data, data will be stripped of identifiers such as name, date of birth, medical record number, and any other information that could be used to identify participants and will be fully de-identified by standards consistent with the Common Rule and HIPAA.

The genotype data will be made publicly available no later than six months after completion of sequencing and analysis for all patients on the study, or the date of initial publication, whichever comes first.

11.0 STATISTICAL CONSIDERATIONS

11.1 Statistical Design and Analysis for the Primary Objectives and Stopping Rules (original cohort)

This is a phase I/II study with two different phases, to determine the maximum effective dose (MED) for CD45RA-depleted DLI when given in the early post-engraftment period in phase 1 and, then to assess efficacy of TCRαβ-depleted progenitor cell graft with additional effective memory T cell DLI defined in phase 1, plus selected use of blinatumomab, in Haploidentical Donor Hematopoietic Cell Transplantation for Hematologic Malignancies in phase 2. The statistical design for phase 1 will be ad-hoc design. The statistical design for phase 2 will evaluate, using a group sequential design, evidence that the new treatment strategy would be expected to result in 1-year EFS in an acceptable rate of patients treated with effective dose determined from phase 1. The

statistical design for the Phase 2 study is an expansion cohort at the effective dose determined in the Phase 1 portion of the study. Those treated at the effective dose in the Phase 1 study will be evaluated for efficacy as part of the Phase 2 study.

Phase 1: Maximum effective dose

For phase 1, a maximum effective dose of CD45RA-depleted DLI is defined as the maximum value of doses that satisfy the rate of patients with their memory T cell count measured at week 4 post-DLI more than 300/uL is more than 50% and the toxicity of grade 3-4 aGVHD is less than 0.2. Only acute GVHD with an onset date within 28 days of the CD45RA-depleted DLI will be considered to be caused by the DLI, all other acute GVHD will be attributed to the transplant in general and not the DLI. Although it is not possible to accurately attribute any GVHD following DLI to the DLI itself versus the overall transplant regimen, we feel that 28 days is an appropriate demarcation for the following reasons: 1) clinical experience shows that positive effects of DLI (such as correction of chimerism) are typically seen at 7 to 14 days after DLI, therefore it is reasonable to postulate that untoward T-cell alloreactivity (such as GVHD) would occur in the same time frame; 2) it is a long standing standard practice across institutions (and in this protocol) to repeat DLI within 4 weeks if the desired efficacy was not attained and if there is no GVHD, indicating that the general consensus based on clinical experience is that the duration of activity following DLI is approximately 4 weeks.

We note that for one specific dose level, the actual dose re-infused to the patients may be slightly different from the target dose. However, per intent to treat, the patients will still be evaluable for that specific dose level on this study. To determine the MED of CD45RA-depleted DLI, we will start to treat patients from dose level 1 and will enroll a cohort of 10 patients at each dose level (See table below for each dose level). We conclude that a dose level X is the MED only if it satisfies 1) among a cohort of 10 patients are treated at dose level X, if 6 or more patients with their memory T cell counts are ≥ 300/uL at week 4 post-DLI and 7 or more patients without grade 3-4 aGvHD, 2) dose level X+1 is too toxic (≥four patients experience grade 3-4 aGVHD) or not efficacious (<6 patients with their memory T cell counts are ≥ 300/uL). Then this MED will be assessed for efficacy and toxicity in phase 2.

Dose level	CD3+ dose
0	1x10^5/kg + MMF through Day +60
1	1x10^5/kg
2	1x10^6/kg
3	1x10^7/kg

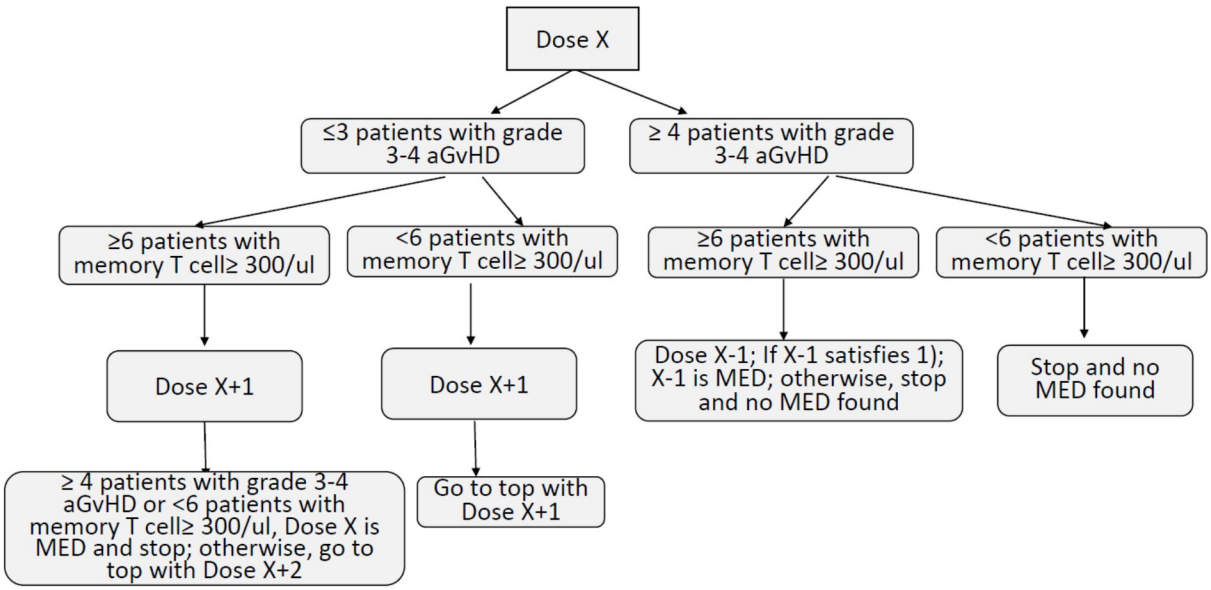
There is no preliminary or published literature estimating the rates of patients with grade 3-4 aGvHD and with memory T cell count ≥300/uL post DLI for the proposed four dose levels, thus we considered two scenarios of projected multinomial probability distribution for grade 3-4 aGvHD and memory T cell count ≥300/uL for four dose levels as pair 1 of [0.05, 0.1, 0.15, 0.25] and [0.2, 0.4, 0.8, 0.9] and pair 2 of [0.05, 0.1, 0.15, 0.4] and [0.2, 0.4, 0.8, 0.9] in our simulations below. If there is no correlation between event of grade 3-4 aGvHD and event of memory T cell counts are ≥ 300/uL, the empirical probabilities that dose levels 2 and 3 would be MED are 0.21 and 0.74 for pair 1 and 0.58 and 0.35 for pair 2 using the diagram below, based on 1000 simulations, respectively.

Similarly, if there is some mild correlation of 0.2 between grade 3-4 aGvHD and memory T cell count ≥ 300/uL, the corresponding simulated probabilities were similar with less than 2% difference. This was obtained using rmvbin function in bindata package to simulate the correlated multivariate binary random variables. This is a phase I part to determine MED, thus, this calculation is only for illustrative purpose and the proposed determination rule is "ad hoc" in nature. However, it was deemed reasonable in PI's evaluation.

Otherwise, diagram below shows that at the current dose level X, if four or more patients experience grade 3-4 aGVHD but there are still ≥6 patients with their memory T cell counts are more than 300/uL at week 4 post-DLI, then this level of CD45RA-depleted DLI is too toxic and we will move one dose level down X-1 if level X-1 is not investigated; otherwise, Dose X-1 is MED.

Otherwise, if less than four patients experience grade 3-4 aGVHD but there are 5 or less patients with their memory T cell counts are less than 300/uL at week 4 post-DLI, then this level of CD45RA-depleted DLI is not efficacious and we will move one dose level up X+1;

Otherwise, if at any time point, four patients experience grade 3-4 aGVHD but there are 5 or more patients with their memory T cell counts are less than 300/uL at week 4 post-DLI, then this level of CD45RA-depleted DLI is too toxic and not efficacious; we will stop and conclude that no MED of CD45RA-depleted DLI is determined if the previous dose does not meet condition 1) above.



If a patient dies from TRM or disease progression within 4 weeks post DLI, it will be counted as a failure in terms of the memory T cell count ≥ 300/uL at week 4 post-DLI.

Phase 2: Efficacy and stopping rules

For phase 2, based on HAPNK1 data frozen on 01/04/2018, 30 out of 36 non-refractory patients without any events (relapse or death) in the first 1 year post transplantation in the standard arm of HAPNK1 (EFS rate is 0.83 (95% CI: 0.67, 0.94) based on exact binomial distribution) (Figure in section 2.2). It may further be noted that on HAPNK1 standard arm there was 8 patients censored within 1 year follow-up because they have not finished their 1-year follow-up. Thus, with assumption of no censoring expected during 1-year time period we can approximate the EFS at 1-year using Binomial distribution. That is, each patient will be followed for one-year and will be counted as a success if the patient survived without any events (death due to any cause or relapse). If any patient is lost follow up within 1 year, we will count it as a failure for EFS. Denote p be the proportion of patients surviving at 1-year without these events mentioned. Although the final EFS number may be slightly smaller after all 8 censored patients have been followed for 1 year, based on the data above, we have assumed an EFS rate of 0.83. Using a one sample test with four looks based on a gamma family spending functions with parameter $\gamma = 1$ for assessing a single proportion with H0: $p \geq 0.83$ vs H1: $p < 0.83$, East 6 gives 85.6% power to detect a decline from 83% to 70% with 0.15 of type I error rate and a sample size of 50 patients. Based on this design, if we observe 6 failures in the first 20 patients, 9 failures in the first 30 patients, 11 failures in the first 40 patients, or 13 failures in the 50 patients within the first one year post transplantation, this would suggest that the proposed treatment approach is inferior.

#Patients	Cum α Spent	Boundary	#Events
20	0.078	0.711	≥6
30	0.107	0.728	≥9
40	0.131	0.744	≥11
50	0.150	0.754	≥13

In addition, we will closely monitor the trial for early excessive toxicities in terms of the safety of Blinatumomab administration, severe acute GvHD (aGvHD), and therapy related mortality (TRM). TRM is any death in remission and related to protocol therapy. Safety of Blinatumomab administration, aGvHD, and TRM will be monitored for 100 days from the date of transplantation. These endpoints will be monitored independently. A patient who experiences grade 3-4 aGVHD and the TRM will count towards both rules.

Below is toxicity monitoring related to Blinatumomab administration. If the drug is held for more than 2 weeks, it will be permanently discontinued. To assure the safety of Blinatumomab administration on this study, if two or more out of the first 6 patients experience Blinatumomab permanent discontinuation due to toxicity, then the study will be placed on hold and the Blinatumomab regimen will be adjusted to a lower dose and/or

premedication will be added. If less than 2 out of the first 6 recipients experience Blinatumomab permanent discontinuation due to toxicity, then the remaining CD19+ recipients may continue to receive Blinatumomab at the proposed dosing. The investigators expect that there will be 15 to 20 patients with a CD19+ leukemia in the 50 or 70 planned recipients (see below).

Out of 34 patients on the standard arm of HAPNK1 patients, 7 patients experienced grade 3-4 aGVHD within 100 days post transplantation (0.206, 95% CI: 0.087~0.379 based on exact binomial distribution). Thus, consideration to stopping/amending the trial would be given if there is evidence suggesting that the rate of grades III-IV aGVHD in 100 days post transplantation is greater than 20%. The planned interim evaluation time points and stopping rules are provided in Table below. This plan is based on one sample test with four looks based on a gamma family spending functions with parameter $\gamma = 2.5$ to reject a high rate of grades III-IV aGVHD of 30% with a type I error rate of 0.2. Based on the Table, if we observe 6, 9, 12, and 15 patients with grade III-IV acute GVHD within the first 100 days post transplantation in the first 20, 30, 40, and 50 evaluable research participants treated, respectively, then we will temporarily halt the trial and consider amending the protocol. Based on these stopping rules, if the true grade 3-4 aGVHD is 0.4, the respective probability of stopping the trial early for excess of grade 3-4 aGVHD would be 0.87, 0.91, 93%, and 0.95. It may be noted that the above stopping rules are "ad hoc" in nature. However, the proposed stopping rules were deemed reasonable in PI's evaluation.

Stopping Rules for Toxicity of Grade III-IV aGVHD within the first 100 days post transplantation.

#No. of Research Participants Enrolled	**Cum α Spent**	**Boundary**	**# Grade 3-4 aGVHD Observed**
20	0.138	0.298	≥6
30	0.169	0.293	≥9
40	0.188	0.286	≥12
50	0.200	0.283	≥15

Out of 34 patients on the standard arm of HAPNK1 patients, 1 patients experienced TRM within 100 days post transplantation (0.03, 95% CI: 0.001~0.15 based on exact binomial distribution). There, besides the stopping rules for monitoring grade 3-4 aGVHD, we will closely monitor the trial for too much TRM, that is, if there are 5 or more patients experience TRM within 100 days post transplantation in the first 25 patients or 8 or more patients experience TRMs in the 50 patients, the study will be paused and assessed for potential changes to the treatment. Based on these stopping rules, if the true TRM rate is 0.2, the respective probability of stopping the trial early for excess of TRM would be

0.58 and 0.81, respectively. It may be noted that the above stopping rules are "ad hoc" in nature. However, the proposed stopping rules were deemed reasonable in PI's evaluation.

With this study plan, the minimum number of patients (whom received protocol defined dosing of CD45RA-depleted DLI and are evaluable at the 4 week time point post DLI) needed for the Phase I portion of the study would be 10 on dose 1. The maximum number of patients (whom received protocol defined dosing of CD45RA-depleted DLI and are evaluable at the 4 week time point post DLI) for Phase I would be 30 patients (10 patients on each dose level 1-3). The Phase II expansion cohort will include 40 additional patients (are evaluable at the 1-year time point post transplantation) treated at the effective dose of CD45RA-depleted DLI determined in the Phase I trial. So the total number of patients for the phase I of the study would be minimum of 10 and maximum of 30. Thus, for both phase 1 and phase 2 of the study, we will need in total a minimum of 50 patients and a maximum of 70 patients.

Based on our St. Jude protocol HAPNK1, the total 34 standard risk patients were enrolled on the study in three years and 3 months with excluding 14 months temporary pause for accrual. With an estimated accrual rate of about 10 participants per year, the study is expected to last for a minimum of 5 years to enroll 50 patients and a maximum of 7 years to enroll 70 patients.

After the study is finished, for the second primary objective, the number of patients without events of relapse and death along with 95% Blyth-Still-Casella confidence interval will be estimated based on the binomial distribution. In addition, to account for potential lost follow-up, the Kaplan-Meier estimate of EFS along with its standard error will also be calculated, where EFS = *min* (date of last follow-up, date of relapse, date of death due to any cause) – date of transplant, and all participants surviving at the time of analysis without events will be censored. As a secondary analysis, we will also perform comparisons of EFS of the study cohort with the standard risk patients in HAPNK1 cohort. The EFS will be compared using the log-rank test method based on a two-sided test.

We propose to analyze and report all outcomes from this original cohort after the primary objective follow-up is complete: when the last evaluable participant enrolled on the original cohort has been followed for one-year post transplant.

11.2 Statistical Analysis for Secondary Objectives (to be done for each cohort individually)

The final results of these secondary objectives are expected to be available when the last evaluable participant has been followed for one-year post-transplant.

11.2.1 Assess the safety and feasibility of the addition of blinatumomab in the early post-engraftment period in patients with CD19+ malignancy.

Giving blinatumomab in the early post-transplant period is novel, and the safety and feasibility of this approach is unknown. Therefore, we will use an ad hoc

design very similar to to the traditional 3+3 phase I approach to describe toxicity and assess safety in many pediatric oncology trials. As described in section 11.1 above, if two patients out of 6 experience blinatumomab permanent discontinuation due to toxicity (as instructed in section 4.2), then the study will be placed on hold and the blinatumomab regimen will be adjusted to a lower dose and/or premedication will be added. If at least 5 of 6 patients are able to successfully receive at least 21 of planned 28 days of blinatumomab without permanent discontinuation, then the addition of blinatumomab in the early post-engraftment period will be considered safe and feasible, and dosing will continue per protocol.

11.2.2 Estimate the incidence of malignant relapse, EFS and OS at one-year post-transplantation

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

11.2.3 Estimate the incidence and severity of acute and chronic GVHD

The cumulative incidence of acute and chronic GVHD will be estimated using Kalbfleisch-Prentice method. Death without acute and chronic GVHD is the competing risk event. The severity of acute GVHD and chronic GVHD will be described. The analysis for this objective will be performed when the last evaluable participant has been followed for 1 year post-transplant.

11.2.4 Estimate the rate of transplant related mortality in the first 100 days after transplantation

The cumulative incidence of transplant related mortality will be estimated using the same method as used in evaluating Objective 11.2.2. Deaths before day 100 because of other reasons are the competing risk events. The analysis for this objective will be performed when the last evaluable participant has been followed for 100 days post-transplant.

11.2.5 To measure and describe the pharmacokinetics of rabbit ATG in HCT recipients on this study.

Pharmacokinetic analysis of rabbit ATG will be performed in the Department of Pharmaceutical Sciences using standard methods.

11.3 Analysis for Exploratory Objectives (to be done for each cohort individually)

The final results of these exploratory objectives are expected to be available when the last evaluable participant has been followed for one-year post-transplant.

11.3.1 Record immune reconstitution parameters, including chimerism analysis, quantitative lymphocyte subsets, T cell receptor excision circle (TREC) analysis, V-beta spectratyping, and phenotype and functional analysis of reconstituted lymphocyte subsets.

All immune reconstitution measures will be descriptively analyzed.

11.3.2 Describe the use of additional CD45RA-depleted DLI for recipients who have <100% donor chimerism, viral reactivation or infection, evidence of disease/recurrence or poor immune recovery (such as lymphopenia). Assess and record efficacy of CD45RA-depleted DLI for these conditions, and all adverse events that are related to CD45RA-depleted DLI.

The use of additional CD45RA-depleted DLI for recipients who have <100% donor chimerism, viral reactivation or infection, evidence of disease/recurrence or poor immune recovery (such as lymphopenia) will be recorded, including dose given, date given, and indication for use and and will be assessed descriptively. To assess efficacy, the resolution of viral infection, resolution of molecular or frank disease, or the correction of severe lymphopenia will be noted. All adverse events that are at least possibly related to CD45RA-depleted DLI and any episode of GVHD will be recorded and will be assessed descriptively.

11.4 Statistical analysis specific to the Early DLI cohort

11.4.1 Early DLI cohort Primary objectives

The early DLI cohort will recruit and transplant 30 patients with the primary objective to assess the feasibility and safety of early CD45RA-depleted DLI administration. We will restart the enrollment count at the time of the first patient enrolled onto the early DLI cohort, and these patients will be summarized separately from the previous cohorts for monitoring and analysis.

Feasibility will be measured by the days from HCT to DLI infusion in the early DLI cohort with the goal to reduce the mean days to DLI infusion by 14 days from the original phase 2 HAP2HCT cohort (mean: 32.2 days, SD: 8.0). This endpoint will be reported descriptively. There will be no stopping rule for this objective.

Safety will be measured by the occurrence of grade III-IV aGVHD in the first 100 days after transplant, and the proposed regimen will be declared safe based on the stopping rule proposed in the table below. This rule was constructed to have a high probability of detecting a grade III-IV aGVHD rate of 28% (which is consistent with the HAPNK1 100-day cumulative incidence estimate) and a low probability of triggering the stopping rule at an 8% rate (consistent with the observed 4 events within 100 days in the original HAP2HCT cohort) using the exact binomial distribution. The 100-day cumulative incidence of grade III-IV aGVHD will be estimated using the Kalbfleisch-Prentice method and reported

with a 95% confidence interval. Death without grade III-IV aGVHD is the competing risk.

Stopping rule for Early DLI cohort based on occurrence of 100-day grade III-IV aGVHD

	Analysis	**Cumulative sample size**	**Cumulative aGVHD III-IV event threshold**	**True 100-day aGVHD III-IV rates**						
				0.08	**0.1**	**0.15**	**0.2**	**0.25**	**0.28**	**0.3**
Probability of crossing the threshold at a given analysis	Interim	10	3	0.04	0.07	0.18	0.32	0.47	0.56	0.62
	Final	30	7	0.00	0.01	0.07	0.17	0.25	0.26	0.26
Probability of declaring the treatment acceptable			≤ 6 events, declare treatment acceptable	0.96	0.92	0.75	0.50	0.28	0.17	0.12

We do not plan to pause enrollment for the interim analysis, anticipating that the risks due to delaying transplantation for eligible subjects are higher than the risks of grade III-IV aGVHD for patients enrolled while the first 10 transplanted patients are still in their 100-day safety follow-up windows. With the expected accrual rates, we also expect very few patients to be enrolled while the first 10 patients are in their safety follow-up. After the 10th transplanted patient completes their safety follow-up, the cumulative number of patients experiencing grade III-IV aGVHD will be compared to the stopping rule. Any patients after the 10th transplanted patient will not be included for this calculation, but their available data will be summarized separately and reported for monitoring. If the interim monitoring threshold is met, the trial will be suspended temporarily, and study therapy will be reviewed by the study team and appropriate regulatory committees. Based on this review, the study may resume as written, with modifications, or be terminated. If the trial does not meet the monitoring threshold, then enrollment will continue as planned.

The planned sample size of 30 patients has high power (>99%) to detect a 14 day decrease in mean days to DLI infusion using a one-sample t-test at the one-sided 5% level with a null hypothesis that the early DLI cohort only reduces the mean time to DLI by 7 days or less (assuming an identical SD as the original cohort). The sample size is also informed by feasibility based on the original cohort accrual and with the goal to generate adequate data for the safety and exploratory objectives.

Based on the experience with the original cohort, we anticipate very few patients will be unable to receive DLI, and patients who fail to receive DLI will not be replaced.

11.4.2 Additional Early DLI cohort Exploratory objectives

11.4.2.1 Assess the degree of T-cell expansion in the early postHCT period and at 4 weeks post-DLI in the Amendment 5 early DLI cohort compared to the original cohort.

T-cell expansion at 4 weeks post-DLI and throughout the early postHCT period will be descriptively compared between patients transplanted on the original cohort and those transplanted on the early DLI cohort.

11.4.2.2 Assess the cumulative incidence of composite viremia (including CMV, EBV, and adenovirus) in the Amendment 5 early DLI cohort compared to the original cohort; and assess the duration of CMV viremia in the Amendment 5 early DLI cohort compared to the original cohort.

The cumulative incidence of composite viremia (including CMV, EBV, and adenovirus) will be estimated using the Kalbfleisch-Prentice method with death without composite viremia as the competing risk. The difference in 60-day cumulative incidence of composite viremia between the Early DLI and original cohorts will be computed and reported with a 95% confidence interval.

Among the patients experiencing CMV viremia, the duration of CMV viremia will be measured as the days from CMV viremia onset until resolution. This will be compared descriptively between the early DLI and original cohorts.

There will be no stopping rule for the exploratory assessments.

12.0 OBTAINING INFORMED CONSENT

12.1 Informed Consent Prior to Research Interventions

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

12.2 Consent at Age of Majority

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

12.3 Consent When English is Not the Primary Language

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

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APPENDIX A:

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

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

APPENDIX B

CRITERIA FOR ACUTE AND CHRONIC GVHD EVALUATION

The St. Jude Department of BMTCT Clinical Research Office standard operating procedure for the evaluation of acute GVHD (SOP 20.01 Acute Graft-versus-Host Disease) and the evaluation of chronic GVHD (SOP 20.02 Chronic Graft-versus-Host Disease) will provide guidance on the evaluation, collection and reporting of acute and chronic GVHD for this clinical trial. The current version of this document, as well as ongoing updates, can be found on the BMTCT Clinical Transplant Program intranet page.

APPENDIX C

CRITERIA FOR ADVERSE EVENT (AE) EVALUATION AND REPORTING

The St. Jude Department of BMTCT Clinical Research Office standard operating procedure for the documenting and reporting of adverse (SOP 10a Capturing and Reporting of Adverse Events for HPC Transplant Patients) will provide guidance on the evaluation, collection and reporting of adverse events for this clinical trial. The current version of this document, as well as ongoing updates, can be found on the BMTCT Clinical Transplant Program intranet page.

APPENDIX D

Recommended testing and evaluation schedule for Transplant Recipient

STANDARD OF CARE STUDIES	SAMPLE	VOLUME	PRE	PRE DLI	MONTH 1	4 weeks post DLI	MONTH 2	MONTH 3	MONTH 6	MONTH 12
Pregnancy Test	PB	2 ml	X		As clinically indicated					
Physical Exam	N/A	N/A	X		Weekly				X	X
GVHD Assessment	N/A	N/A			Weekly					
CBC with diff.	PB	0.5-2 ml	X		Daily until engrafted, then weekly				X	X
Chemistry	PB	0.25-2ml	X		Weekly				X	X
Viral surveillance (BMTPCR)	PB	4 ml	X		Weekly				As clinically indicated	
Chimerism	PB	1-2 ml			Weekly upon engraftment				X	X
	BM	2 ml			X			X		X
Disease Status Evaluation	N/A	N/A	X		X			X		X
MRD Bone Marrow	BM	3 ml	X		X			X		X
Lymphocyte Subset Study	PB	2.5-4 ml	X		X		X	X	X	X
					Weekly during Blinatumomab administration					
Lymphocyte Subset with RARO	PB	2.5- 4 ml		X		X				
Quantitative Immunoglobulins	PB	2 ml	X					X		X

- The information derived from or noted on the physical examinations, standard tests, and other assessments that comprise standard of care for recipients are not required to be transcribed onto case report forms and/or entered into the database. In reference to section 6.1 Evaluations, the above-indicated follow-up regimen for these evaluations is guided by the SOPs of the Department of BMTCT, for recipients of allogeneic stem cell transplantation. As these evaluations are considered standard clinical care (non-research), variations in frequency (more or less frequent) of these evaluations can occur due to the participant's current clinical condition and will not be noted as protocol deviations with exception of the pre and post DLI Lymph Subset with RARO..
- Disease status evaluations/BM testing results obtained prior to enrollment may be used for the baseline/pre-infusion assessments.
- Additional chimerism testing may be required as clinically indicated and described in Section 6.3.
- Lymphocyte subset studies may be omitted without variance when the absolute lymphocyte count (ALC) is zero.
- In the event of graft failure/rejection, the post failure/rejection bone marrow, chimerism and several applicable immune studies would not be clinically indicated and these studies may be held.

APPENDIX D (continued)

Immune reconstitution testing and evaluation schedule for Transplant Recipient

RESEARCH STUDIES	SAMPLE	VOLUME	PRE	MONTH 1	MONTH 2	MONTH 3	MONTH 6	MONTH 12
VBETA/TREC RESEARCH	PB	8 mL				X	X	X
LYMPHOCYTE RESEARCH	PB	12 mL	X	X	X	X	X	X
PHENOTYPE RESEARCH	PB	5 mL	X	X	X	X	X	X
IR-PHENOTYPE (Youngblood Lab*)	PB	5 mL	X	X	X	X	X	X
T-FUNCTION (Thomas Lab*)	PB	5 mL	X	X	X	X	X	X
	BM	1 mL	X	X		X		

- VBETA/TREC Research and Lymphocyte Research results will be maintained in the Immune Monitoring Core database.
- Phenotype Research results will be maintained in the Flow Core database.
- For RESEARCH studies, the posted volumes are the minimum volumes required to perform the respective protocol evaluations.
- When blood volumes exceed established safety parameters (3% of patient blood volume in 24hr) priority for blood draws go to clinically necessary labs first, then research labs in order from top to the bottom of this table. However, for the pre-transplant time point the priority for research labs is from the bottom to the top of this table. Any omissions in testing due to this safety parameter will not constitute a protocol deviation or variance.
- The PRE-transplant bone marrow for T-FUNCTION is an optional test that may be omitted at the discretion of the PI.

* Research testing results will be maintained in a secured database in the respective co-investigator laboratory database.

APPENDIX D (continued)

ATG PK Testing for original cohort

OPTIONAL Evaluation for ATG PK Testing RESEARCH STUDY	SAMPLE	VOLUME	Day -3 (Pre 3rd dose)	Day -3 (post 3rd dose)	D -1	D 0	D +2	D +4	D +7	D +14	D +21
ATG PK	PB	2 mL	X	X	X	X	X	X	X	X	X

- Sample collection date may be adjusted ±2 days as needed (such as to accommodate blood volume concerns, line access issues, etc.) The ±2 days adjustment will not apply to the Day -3, -1, 0, +2 and +4 samples.

ATG PK Testing for early DLI cohort

OPTIONAL Evaluation for ATG PK Testing RESEARCH STUDY	SAMPLE	VOLUME	Day -10 (Pre 3rd dose)	Day -10 (post 3rd dose)	D -8	D -7	D -5	D -3	D 0	D +7	D +14
ATG PK	PB	2 mL	X	X	X	X	X	X	X	X	X

- Sample collection date may be adjusted ±2 days as needed (such as to accommodate blood volume concerns, line access issues, etc.) The ±2 days adjustment will not apply to the Day -10, -8, -7, and Day-5 samples.

APPENDIX D (continued)

Research testing for DONOR

Prior to initial stem cell collection procedure:

OPTIONAL research immune studies testing schedule

Evaluation	*Volume Requirement*
Flow cytometry enumeration	Lymphocyte Subset Study = 4 mL
Thymic output and T cell repertoire	VBETA/TREC Research = 17 mL
Donor baseline lymphocyte number and function (Immune Monitoring Core)	Lymphocyte Research = 12 mL
Donor baseline phenotype number and function (Flow Core)	Phenotype Research = 5mL
Donor baseline immune function (Youngblood Lab*)	IR-PHENOTYPE = 5mL
Donor baseline immune function (Thomas Lab*)	T-FUNCTION = 5mL

- All donor research testing to be collected prior to growth factor administration and progenitor cell collection. These optional research tests may be collected at separate times.
- Research testing results will be maintained in a secured database in the respective co-investigator laboratory database.

APPENDIX D (continued)

Research Study Evaluation Target Windows

Several laboratory tests can only be processed on weekdays; therefore, if the scheduled evaluation falls on a weekend, or during a holiday period, an adjustment in the follow-up visit is expected and would not be noted as a protocol variation. Additionally, in order to accommodate such logistical constraints, evaluation/collection dates of all protocol assessments (required and optional research), may be performed within a reasonable window of the intended date following the guidelines provided in the table below:

If the Planned Evaluation Time Point is:	Window
Weekly	± 3 Days
Month 1	Week 2 to Week 6
Month 2	Week 7 to Week 11
Month 3	Week 12 to Month 4
Month 6	Month 5 to Month 7
Month 9	Month 8 to Month 10
Month 12	Month 10 to Month 14

APPENDIX E

The St. Jude Department of BMTCT Clinical SOPs for standard of care for all allogeneic stem cell infusion recipients and stem cell donors will provide guidance on the evaluation, ongoing clinical care and follow up for this clinical trial. The current versions of these SOPs, as well as ongoing updates, of these documents can be found on the BMTCT Clinical Transplant Program intranet page.