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A REDUCED INTENSITY CONDITIONING REGIMEN WITH CD3-DEPLETED HEMATOPOIETIC STEM CELLS TO IMPROVE SURVIVAL FOR PATIENTS WITH HEMATOLOGIC MALIGNANCIES UNDERGOING HAPLOIDENTICAL STEM CELL TRANSPLANTATION

IDE #9162

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STUDY SUMMARY

Allogeneic hematopoietic stem cell transplantation (HSCT) has improved the outcome for patients with high-risk hematologic malignancies. However, many patients do not have a matched sibling donor available or are unable to identify an acceptable unrelated donor in a timely manner. Therefore, many of these patients are unable to undergo allogeneic transplantation, which is the preferred or sole curative treatment for their disorder. Haploidentical (HAPLO) HSCT, using a mismatched family member, is another transplantation option for these patients. While this method of transplantation has proven curative in many patients, there are significant transplant-related issues that require further study.

Graft-versus-host disease (GVHD), infections due to delayed immune reconstitution, regimen-related toxicity, posttransplant lymphoproliferative disorder (PTLPD), and relapse have been and continue to be barriers to successful haploidentical transplantation, resulting in death or a poor quality of life in transplant recipients. To reduce the incidence of severe GVHD, patients undergoing HAPLO HSCT must receive grafts depleted of T lymphocytes. In prior studies at our institution, we demonstrated that HAPLO HSCT with CD34+ selected or CD3-depleted grafts is feasible, resulting in prompt engraftment with low rates of PTLPD, which has been treatable in the majority of cases.

In our first clinical trial, HAPSCT, we used a myeloablative total body irradiation (TBI)-based regimen for children and young adults with high-risk hematologic malignancies undergoing HAPLO HSCT. All participants engrafted promptly and survival rates were promising, but regimen-related toxicity and disease recurrence were significant problems. Immune reconstitution was delayed, and viral reactivation occurred commonly. A concurrent trial (REFSCT) using a reduced intensity conditioning regimen for patients undergoing second allogeneic HSCT or with refractory disease, demonstrated prompt engraftment with low rates of severe GVHD and regimen-related toxicity. When compared with the high-risk patients undergoing HAPLO HSCT, the refractory group had more rapid immune reconstitution with lower rates of viral reactivation. Based on these observations, our next generation of clinical trials, HAPREF, used a reduced intensity regimen for both patient populations (high-risk and second HSCT/refractory) combined with a higher CD3+ graft content. However, in this latter study intentionally employing grafts with higher CD3+ graft content to increase anti-leukemia effects, we observed that the higher CD3+ graft content was associated with higher rates of overall grade III-IV acute GVHD.

In this clinical trial, we aim to improve event-free survival by reducing GVHD and regimen-related mortality by increasing the tempo of immune reconstitution using a reduced intensity-conditioning regimen. Transplant recipients will receive a mismatched family member donor graft partially depleted of T lymphocytes using the investigational CliniMACS device and the anti-CD3 antibody, with a limited number of CD3 cells in the graft. For participants who meet the maximum CD3 dose on the first day of collection, the second day of collection will be depleted of T lymphocytes by CD34+ selection using an anti-CD34 antibody and the CliniMACS system. This product manipulation will be used to derive an allogeneic graft with an acceptable CD3 content and maximal CD34 content from cytokine-mobilized peripheral blood stem cell products. A reduced intensity-conditioning regimen without TBI will be employed in an effort to reduce regimen-related toxicity and mortality.

This protocol will describe event-free, disease-free, and overall survival following HAPLO HSCT with a graft partially depleted of CD3+ cells using the CliniMACS system. Secondly, this trial will define a platform for performing HAPLO HSCT safely so that future studies of posttransplant immunomodulation designed to improve immune reconstitution and to generate anti-leukemia effects can be employed. Additional goals of this protocol are to estimate the rates of acute and chronic GVHD, graft failure, and peri-transplant morbidity and mortality in this group of research participants. We will also explore defined biologic markers as predictive factors for the development of acute and chronic GVHD in these participants. Ultimately, this study will serve as a foundation on which to build future studies of immunomodulation and immune reconstitution in the posttransplant setting with the ultimate goal of identifying strategies that significantly decrease disease recurrence, enhance immune reconstitution, and improve overall treatment outcome for patients with high-risk hematologic malignancies undergoing HAPLO HSCT.

Identification of the St. Jude protocols referred to throughout document:

HAPSCT

Title: Haploidentical Stem Cell Transplantation Utilizing Purified CD34+ Hematopoietic Cells for Patients with Hematological Malignancies

Total number of transplant recipients = 27

Arms/strata: Arm A = CD34 positive selection - N = 7
Arm B = CD3 negative selection - N=20

Date of first transplant: 05/2002 Date of last transplant: 06/2005

REFSCT

Title: Haploidentical Stem Cell Transplantation Utilizing T-Cell Depletion as Therapy for Patient with Refractory Hematological Malignancies

Total number of transplant recipients = 25

Arms/strata: Not applicable, 1 arm/not stratified

Date of first transplant: 06/2003 Date of last transplant: 07/2005

HAPREF

Title: Haploidentical Hematopoietic Stem Cell Transplantation Utilizing Partial T-Cell Depletion as Immunotherapy for Hematologic Malignancies.

Total number of transplant recipients = 17

Arms/strata Arm A = High-Risk Disease - N = 9 Arm B = Refractory Disease - N = 8

Date of first transplant: 09/2005 Date of last transplant: 03/2006

1.0 OBJECTIVES

1.1 Primary objective:

1.1.1 To assess if the event-free survival at one-year posttransplant for research participants with high-risk hematologic malignancies can be improved following HAPLO HSCT using a graft depleted of CD3+ cells *ex vivo* and a reduced intensity-conditioning regimen.

1.2 Secondary objectives:

1.2.1. To estimate the one-year overall survival (OS) and disease-free survival (DFS) for research participants who receive this study treatment.

1.2.2 To estimate the cumulative incidence of relapse for research participants who receive this study treatment.

1.2.3 To estimate the rate of overall grade III-IV acute GVHD, and the rate and severity of chronic GVHD in research participants.

1.2.4 To estimate the incidence of non-hematologic regimen-related toxicity and regimen-related mortality in the first 100 days posttransplant.

1.3 Exploratory objectives:

1.3.1 To explore the biologic significance of soluble interleukin-2 receptor and immunologic state (quantitative lymphocyte studies, V beta spectratyping, TREC assay) to predict the development of acute and chronic GVHD in these research participants.

1.3.2 To measure the pharmacokinetics of Campath-1H in pediatric HAPLO HSCT recipients

2.0 BACKGROUND AND RATIONALE

2.1 Introduction

Allogeneic HSCT is curative for many childhood hematologic malignancies that fail standard dose chemotherapy. Unfortunately, many patients do not have an HLA-identical sibling donor or an appropriate matched unrelated donor (MUD) identified.

Furthermore, regimen-related toxicity, acute and chronic GVHD, prolonged immuno-deficiency, and disease recurrence remain significant causes of morbidity and mortality among patients who do undergo transplant. Thus, therapies that reduce regimen-related toxicity, GVHD, and relapse while promoting immune reconstitution may improve disease-free survival and quality of life, allowing this treatment to be extended to all patients who require it. Nearly all patients have a readily available mismatched family member donor. However, T-cell depletion of these grafts, predominantly by negative selection strategies, has been necessary for successful transplantation. Even so, severe GVHD, infections, and PTLPD are significant problems.

Preliminary data suggest that grafts depleted of $CD3^+$ cells engraft promptly in patients receiving stem cell grafts from alternate donors (unrelated or HAPLO family member donors). Therefore, for patients lacking a matched sibling or an available unrelated donor, the transplant program at St. Jude Children's Research Hospital (St. Jude) plans to utilize

grafts containing large numbers of hematopoietic progenitor cells that are depleted of T lymphocytes. The graft will be engineered to the target values using the CliniMACS system to specifically deplete CD3+ T cells using the OKT-3 antibody. This protocol will also address specific issues of engraftment, GVHD, chimerism, regimen-related toxicity, disease recurrence, and posttransplant cellular therapies based on chimerism status.

2.2 Indications for HSCT

Many childhood leukemias that cannot be cured with chemotherapy alone can be effectively treated by allogeneic bone marrow transplantation.[1,2] Moreover, for patients with chronic myeloid leukemia (CML), allogeneic HSCT is the only modality of treatment that is curative. Several studies have demonstrated that transplantation from a matched sibling donor reduces the risk of relapse in patients with acute myeloid leukemia (AML) in 1st or 2nd remission and in patients with acute lymphoblastic leukemia (ALL) in 2nd remission. However, although effective at eradicating disease in this context, improvements in survival are limited by the morbidity and mortality associated with the procedure.[3-14] Nevertheless, when marrow transplantation is undertaken using an HLA-matched sibling as the donor, multi-institutional studies have demonstrated that the procedure is curative in 60-80% of patients with CML, 50% of patients with standard or high-risk leukemia (AML or ALL in 1st remission), 30% of patients with myelodysplasia, and 20-30% of patients with poor-risk leukemia (AML or ALL in second or subsequent remission, AML or ALL in relapse and secondary leukemia).[10,12,15-17,] These data have been corroborated by our single institution studies at St. Jude, with standard-risk recipients achieving an 81% 2-year disease-free survival (DFS), and a 30% DFS being observed in recipients at high-risk of relapse.[18]

Patients with non-Hodgkin lymphoma or Hodgkin disease who recur after autologous HSCT or who have refractory disease are generally not curable with autologous transplantation, salvage chemotherapy, or radiation therapy alone. In addition, some studies demonstrate high rates of morbidity and mortality in lymphoma patients undergoing allogeneic HSCT after failing previous autologous HSCT.[19] These patients typically require more aggressive novel therapies such as allogeneic HSCT, which takes advantage of an allogeneic graft vs. lymphoma effect, while significantly reducing regimen-related toxicities. Preliminary studies in these patient populations are promising, suggesting that following allogeneic HSCT, even those with refractory and multiply relapsed disease can be long-term survivors.[20-24] Allogeneic HSCT also allows the use of other novel therapeutic maneuvers such as the withdrawal of immunosuppression after allogeneic HSCT and donor lymphocyte infusions (DLIs or therapeutic cell, T-cell infusions) to enhance the graft vs. lymphoma effect.

2.3 Hematopoietic stem cell transplantation using alternate donors

Therapeutic options for the majority of patients with leukemia requiring allogeneic HSCT have focused on the use of two groups of alternate donors, given that only approximately 30% of patients have a suitable matched sibling donor.[25] HAPLO donors share a genotypically identical HLA-haplotype and possibly additional phenotypic identity on the unshared haplotype with the recipient.[26]Alternatively, the identification and utilization of unrelated donor bone marrow grafts, termed matched unrelated donors (MUD), who share phenotypic or genotypic HLA identity or near-identity with the recipient has been supported

by the establishment of the National Marrow Donor Program (NMDP)[27]However, many patients do not have an appropriate unrelated donor identified or formally requested for donation in a timely manner. Thus, HAPLO donors are a viable alternative since these family members are highly motivated, easily accessible, and readily available for most patients.

Transplantation with unmanipulated alternate donor stem cell grafts result in a significant increase in the incidence of GVHD and graft rejection (a form of host-versus-graft or HvG disease) when compared to recipients of matched sibling donor grafts, particularly with respect to HAPLO grafts. Several factors influence graft rejection including the degree of immunosuppression of the recipient, the genetic disparity between donor and recipient, and the decreasing numbers of $CD3^+$, $CD34^+$, and total nucleated cells/kg in the graft. In contrast, the incidence of GVHD is not only associated with the degree of donor-recipient genetic disparity but also with the intensity of conditioning, the recipient's age, increasing numbers of $CD3^+$ cells and decreasing numbers of $CD34^+$ cells.[28] Finally, this complex relationship is further compounded by the high-risk nature of the leukemias treated with this approach, as well as with the risk of increasing peri-transplant toxicity as it relates to the intensity of pre-transplant therapy.[27-30]

2.4 The use of T-cell depleted allografts by negative selection

Several approaches have been developed to reduce the incidence of GVHD in the HAPLO donor setting. Because larger numbers of T cells (>3 x 10^6 $CD3^+$ cells/kg) have resulted in grade III-IV acute GVHD with significant morbidity and mortality,[28] investigators have utilized methods to remove T cells from grafts. Methods of T-cell depletion are based predominantly on negative selection strategies. These include physical methods such as T-cell specific lectins in association with sheep RBC rosetting or counterflow elutriation.[31-33]An alternate approach is the use of T-cell specific monoclonal antibodies in association with complement, immunotoxins or magnetic beads.[34-45]These approaches in reducing T cell number have resulted in an approximately 26 - 35% disease-free survival in high-risk leukemia, with a 16-39% incidence of grade II-IV GVHD and a 5-10% non-engraftment rate.[27,46-48]However, despite the use of various preparative regimens in this patient group, there have been no demonstrable differences in outcome by the type of regimen employed. Overall, 30-45% of patients succumb to regimen-related toxicity with an additional 30-40% dying from progressive disease or recurrence.[26-28,46-53]

Our institutional experience at St. Jude with T-cell depleted MUD-HSCT (HUD, MISMUD) demonstrate that outcomes following MUD-HSCT (including both 5/6 and 6/6 MUD as a single group) are similar to those from HLA-identical siblings.[18] These studies were based on the infusion of an allograft depleted of T cells for unrelated donors, primarily utilizing a CD6/CD8 monoclonal antibody/complement cocktail. We observed a significant reduction in the incidence of GVHD-related complications utilizing this approach, the incidence of grade II-IV acute GVHD being 22% with a 19% incidence of chronic. Coincidentally, our graft failure incidence remains quite low (less than 5%) in recipients of alternate donor grafts.[54] However, at our institution, HAPLO donor product recipients continued to have inferior overall survival (OS) and DFS when compared to MUD recipients even when adjusted for the number of recipients with high-risk disease, reflecting the historical observation that donor source is related to recipient outcome.[55]

In addition to regimen-related toxicity and disease recurrence, the use of T-cell depleted grafts is associated with higher rates of graft failure when compared to HSCT utilizing unmanipulated grafts. Indeed, this complication, coupled with a high incidence of GVHD, has hindered the use of the most available group of donors: mismatched family members. Previous efforts to overcome this barrier have focused on the eradication of residual host-immunocompetent cells, the probable mediators of rejection. These include highly immunosuppressive chemo-radiotherapy conditioning regimens and/or the *in vivo* administration of ATG or monoclonal antibodies.[56-59]However, these approaches increase the risk of posttransplant infectious complications. An alternate strategy is derived from studies by Handgretinger and other investigators, suggesting that increasing numbers of $CD34^+$ hematopoietic cells may enable the recipient to better tolerate the genetic disparities between self and donor.[60]These results, coupled with studies in the autologous HSCT setting which demonstrate that large numbers of $CD34^+$ cells can be harvested by peripheral blood apheresis, suggest a new approach to achieving stable engraftment with a reduced incidence of peri-transplant toxicity.

2.5 Experience at St. Jude using allogeneic HSCT for patients with high-risk hematologic malignancies

Between the years 1993 to 2000, 249 infants, children and young adults with high-risk hematologic malignancies underwent allogeneic HSCT at St. Jude (Laura Bowman, unpublished data). Donor sources are listed below:

Donor Source	N
Matched sibling donor	84
Unrelated donor matched at 6/6 HLA loci	86
Unrelated donor matched at 5/6 HLA loci	48
Mismatched family member	31

It has been the policy of the Transplant Program at St. Jude that children should receive grafts from an HLA-identical sibling when one is available. Eighty-four of these patients received HLA-identical sibling grafts during the time period. For patients lacking a matched sibling, a search for a MUD was performed. During this time period, acceptable unrelated donors were those matched at 5 or 6 HLA loci. Of these, 86 and 48 patients received a 6/6 and 5/6 unrelated donor graft, respectively. These patients received a graft depleted of T cells using CD6/CD8 monoclonal antibodies plus complement. In recipients of MUD grafts matched at 6 HLA loci, grade 0, I, II, III, and IV acute GVHD occurred in 34, 38, 7, 3, and 4 patients, respectively. Chronic GVHD was seen in 9 of 86 evaluable patients. In patients with lymphoid and myeloid malignancies, the overall survival at one year is 49.2 $\pm$ 10.1% and 55.7 $\pm$ 6.3%, respectively, for recipients of 6/6 MUD grafts.

For recipients of marrow matched at 5/6 HLA loci, grade 0, I, II, III, and IV acute GVHD occurred in 15, 19, 3, 5, and 6 patients, respectively. Chronic GVHD was seen in 9 of 48 evaluable patients. In patients with lymphoid and myeloid malignancies, the overall survival at one year is 36.4 $\pm$ 13.0%% and 37.8 $\pm$ 7.7%, respectively. Thus, the 5/6

MUD transplant recipients had an inferior survival as compared to the 6/6 MUD transplant patients.

During this time period, 31 patients with high-risk hematologic malignancies underwent allogeneic HSCT from HAPLO donors. All patients received the same graft manipulation, conditioning regimen and GVHD prophylaxis as those receiving unrelated donor grafts. Fourteen patients had no acute GVHD, 6 experienced overall acute grade I, 8 grade II, none with grade III, and 3 with grade IV. Four of 16 evaluable patients had chronic GVHD. Of the 8 patients transplanted from donors matched at 3/6 HLA loci, only 1 survives. Of the 8 receiving grafts from donors matched at 4/6 HLA loci, 3 survive. Of 15 patients transplanted from donors matched at 5/6 HLA loci, 5 survive. Patients in the mismatched family member recipient group who have lymphoid malignancies have an overall survival of 22.2 $\pm$ 13.9% at one year posttransplant; those with myeloid malignancies have an overall survival of 34.5 $\pm$ 9.9% at this same one year posttransplant time period.

It is important to note several facts about the patient population undergoing HAPLO HSCT at St. Jude between the years of 1993 to 2000. These patients had high-risk hematologic malignancies and had been heavily pretreated and many had refractory disease. Patients and their health care providers often chose to delay transplant as long as possible due to concerns over morbidity and mortality in those patients having only a HAPLO donor. In addition, patients who had medical conditions or infections, which made allogeneic HSCT with traditional methodologies too risky, were not offered this potentially curative procedure. Lastly, it is important to realize that patients who did not have an unrelated donor identified and confirmed through a registry search within a 90 day period were unlikely to have one identified by prolonging the time required for the donor search. Therefore, the number of patients who actually underwent HAPLO HSCT from 1993 to 2000 is an underestimation of the potential population who would benefit from this approach. Thus, all patients who could potentially be cured by HSCT at our institution during this time period were not able to proceed to allogeneic HSCT if their only donor were a HAPLO. Furthermore, from the donor-recipient match distribution, it is evident that this is a selected population with fewer patients transplanted with donors matched at 3 HLA loci and a preponderance of those transplanted matching at 4 or 5 HLA loci.

2.6 Allogeneic peripheral blood stem cell (PBSC) transplantation utilizing unmanipulated grafts

Initial studies of allogeneic PBSC transplantation have focused predominantly on matched sibling allografts. The first report of allogeneic PBSC transplantation from a matched sibling was published in 1989.[61] Since that time, reports of syngeneic and matched-sibling PBSC transplants, utilizing unmanipulated grafts, have demonstrated a similar outcome to that observed for patients undergoing allogeneic bone marrow transplant (BMT).[61-65]However, several added benefits appear to be inherent to allogeneic PBSC grafts. These include more rapid engraftment and immune reconstitution, decreased treatment-related mortality and the ability to deliver a larger hematopoietic stem cell dose as measured by the number of $CD34^+$ cells/kg infused, when compared to other forms of graft manipulation.[66-72] In addition, recent data suggests that the number of $CD34^+$ cells infused can be related to both the rate of engraftment and peritransplant toxicity.[73]However, GVHD is a concern with this approach

with the unmanipulated graft containing a 10-100 fold higher number of $CD3^+$ cells/kg. Although no significant increase in acute GVHD in patients receiving matched sibling donor grafts has been observed, an increased incidence of chronic GVHD has been reported in this population.[68,74,] Finally, a low number of $CD3^+$ cells in the graft has been identified as the most important factor for graft failure following PBSCT in this sibling donor product recipient population.[75]

2.7 $CD34^+$ positive selection strategies

The CD34 antigen, a cell surface glycoprotein expressed on a small fraction of bone marrow cells, serves as a surrogate marker for the hematopoietic stem cell. This population contains cells capable of long-term hematopoietic reconstitution of all hematopoietic lineages.[76-81]Several approaches have been utilized to perform $CD34^+$ selection. Two of these approaches, the Baxter Isolex and CellPro Ceprate systems, have proven invaluable for $CD34^+$ cell selection and tumor purging of autologous grafts.[81-83]However, these approaches lack the ability to exclude sufficient T cells to allow graft infusion without further graft manipulation.[84]In contrast, the Miltenyi CliniMACS $CD34^+$ selection system allows the isolation of $CD34^+$ cells in a single procedure with minimal contamination by mature T- and B cells.[85,86]In over 70 grafts prepared for infusion, this has resulted in a median $CD34^+$ purity of 97% with a yield of 71%, and an associated 99-99.9% depletion of T cells.[85,86]In contrast, these results are unattainable with other $CD34^+$ selection systems. Thus, the reproducible purification of large numbers of $CD34^+$ cells permits a precise titration of the number of $CD34^+$ and $CD3^+$ cells in the graft, allowing the infusion of a defined graft in all patients.

2.8 Preliminary studies of $CD34^+$ based allografts obtained from alternate donors

Studies of HSCT in the HAPLO setting suggest that removal of T cells reduces the incidence of GVHD significantly. To date, only small studies utilizing $CD34^+$-selected HAPLO grafts have been performed. However, the results are informative and suggest that this approach may be highly beneficial. In patients with high-risk leukemia, Aversa and colleagues have reported successful long-term engraftment with minimal GVHD utilizing $CD34^+$ enriched grafts, purified utilizing positive ($CD34^+$) and negative (lectin agglutination) selection.[84]A disease-free survival of 30% was reported in this study, with a similar incidence of relapse and a 40% incidence of transplant-related mortality.

The Tuebingen group (Handgretinger and colleagues, Tuebingen, Germany) has focused their efforts on optimizing the CliniMACS $CD34^+$ positive selection system. Utilizing predominantly HAPLO grafts and megadose cell therapy ($\geq 1 \times 10^7$ $CD34^+$ cells/kg) to overcome engraftment resistance, they have reported similar preliminary results in a pediatric population.[60,85] They initially studied the utility of highly purified grafts prepared from PBSC products obtained from HAPLO parental donors in a population of patients with high-risk leukemia who lacked matched sibling or matched unrelated donors. Using megadoses of $CD34^+$ cells ($20.7 \pm 9.8 \times 10^6$ CD34+ cells/kg), the Tuebingen group observed a low incidence of graft failure (7%), a median time to engraftment of 11 days, and no severe primary acute grade III-IV GVHD in 39 patients treated. Of note, since acute GVHD was not observed in the first 7 patients treated, the subsequent 32 patients received no pharmacologic GVHD prophylaxis.

Graft failure appeared to be related to ATG-containing regimens and in recipients given fewer than 1.0 x 10^7 $CD34^+$ cells/kg. Two of these patients re-engrafted after a second infusion of a higher dose of HAPLO $CD34^+$ cells following the administration of the pan T cell antibody OKT3 and methlyprednisolone. In this cohort, 33% of patients have relapsed and 10 patients (25%) have died of regimen-related toxicity. Of these 10 deaths, 5% were related to veno-occlusive disease (VOD), 18% due to viral and fungal infections, and 2.5% due to non-engraftment. Interestingly, neither this cohort, nor subsequent patients reported below have experienced Epstein-Barr virus (EBV) PTLPD, a known complication of T-cell depletion methodologies. This observation is consistent with reports of others that this complication is related, at least in part, to the number of mature B cells in the infused graft.

Between 1995 and 2000, the Tuebingen group provided 48 children and adolescents with HAPLO grafts processed on the CliniMACS system. Thirty-eight of these patients had hematologic malignancies and will be discussed more fully in this section (Rupert Handgretinger, unpublished data). Of these 38, 6 patients with ALL in relapse at the time of transplant are excluded to more accurately make comparisons with St. Jude data as these patients are not transplanted at St. Jude. Diagnoses included ALL (5 in CR1, 4 in CR2, 5 in $\geq$ CR3), NHL (1 in CR2, 1 in CR3, 2 with persistent disease), AML (1 in CR1, 1 in CR2, 4 with disease); MDS (4 with disease), and CML (4 chronic phase). No patient developed acute GVHD of greater than grade II and none experienced chronic GVHD. Twelve of these patients are alive disease-free. Twelve died of relapse, 4 of infection, 2 of regimen-related toxicity, and 1 of an accident while in remission. Patients with lymphoid malignancies (ALL, NHL) have an overall survival of 47.7 $\pm$ 12.2% at one-year posttransplant; those with myeloid malignancies (CML, AML, MDS) have an overall survival of 24.5 $\pm$ 10.6% at the one-year posttransplant time period.

Several important facts must be noted with this Tuebingen experience. This patient group was indeed high-risk with the majority having sustained several prior relapses or having active disease at transplant, particularly in those with myeloid diseases. All patients for whom allogeneic HSCT was indicated underwent the procedure; no patients were denied HSCT due to lack of a donor. Furthermore, all patients received HAPLO grafts, with donor and recipients matched at 3/6 HLA loci.

Historically, the most powerful prognostic features of patients undergoing allogeneic HSCT have included leukemia type, disease status at time of HSCT, and donor stem cell source. When comparing overall survival rates in the patients treated at Tuebingen and at St. Jude, several important differences are noted. First, the methods for graft T-cell depletion were different. Second, all patients eligible for transplantation underwent this procedure in Germany, while all referred patients at St. Jude did not. Third, disease status at the time of transplant was significantly different between Tuebingen and St. Jude, possibly due to differences in patient referral patterns and treatment philosophies. For example, in Tuebingen, patients with AML in first CR were not referred for allogeneic HSCT from alternate donors. At St. Jude, patients with myeloid malignancies were referred for transplantation when deemed high-risk, including those in first remission. The converse was true for patients with lymphoid malignancies. Patients at St. Jude were referred for HSCT much later than patients in Tuebingen. These differences in disease status at the time of transplant were quite significant. In fact, they were

sufficiently poor enough to obscure effects of donor source, particularly in the lymphoid disease group. However, for the proposed trial we anticipate that referral patterns will reflect those historically observed at St. Jude.

Based on these encouraging preliminary data, participants in this trial undergoing HAPLO-HSCT will receive partially depleted hematopoietic grafts from HAPLO family member donors depleted of T cells using negative selection. Intravenous Campath-1H will be administered in the conditioning regimen to enhance immunosuppression and decrease the incidence of graft rejection. In patients experiencing mixed chimerism or graft failure, withdrawal of immunosuppression (if applicable) with DLIs and $CD34^+$ boosts/repeat stem cell infusions, respectively, will be utilized to restore hematologic and immunologic function.

2.9 Evaluation and treatment of mixed chimerism and graft failure after $CD34^+$-enriched allografts

Mixed chimerism (10%-95% donor engraftment), graft failure (<10% donor engraftment) and disease recurrence remain common problems after HSCT. The ability to detect these complications and to treat them effectively is an important goal. Preliminary data suggests that monitoring hematopoietic chimerism in a serial fashion may provide a guide for therapeutic intervention.[87-89]For example, in 55 allograft recipients, those with complete donor chimerism experienced a 67% relapse free survival rate. Of these 55, those with increasing donor chimerism experienced 100% survival while recipients with decreasing donor chimerism demonstrated a 10% survival rate.[89] In a similar group of 32 patients receiving allografts for malignant diseases, the risk of developing relapse or graft failure was significantly increased in recipients with mixed chimerism when compared with those with full donor chimerism ($p<0.0005$).[87]

One approach to treating mixed chimerism is the use of posttransplant therapeutic cell, T cell infusions, so called DLIs. This approach has been used extensively by others, and us, and has been successful as a therapeutic strategy.[90]In a pilot feasibility study, the Tuebingen group treated 21 patients considered to be at high-risk of relapse or who showed transient host hematopoietic recovery at various times posttransplant with a CD3 dose between 2.5×10^4 to 1×10^5 $CD3^+$/kg. These infusions were performed once in 9 patients, twice in 7, three times in 4 and four times in 1 patient. Five patients developed grade I-II acute GVHD; only 1 patient developed grade IV disease. Chronic GVHD was induced in 1 patient. It is important to note that the incidence of GVHD was related to T cell dose, with those receiving less than 3×10^4 $CD3^+$ cells/kg being less likely to experience this complication.

This preliminary experience suggests that the related complication, graft failure, may respond to immunosuppressive reconditioning regimens and infusion of a second $CD34^+$-enriched allograft. Of 9 pediatric stem cell recipients receiving highly purified $CD34^+$ grafts, 3 did not engraft and 6 rejected their graft after 21 days. The 4 patients receiving grafts from an unrelated donor were reconditioned with fludarabine (40 mg/m^2/day for 5 days), cyclophosphamide (60 mg/kg/dose) for 1 day and ATG. The 5 recipients of HAPLO related transplants received methlyprednisolone (20 mg/kg/day for 4 days) and OKT3. Following reconditioning, a second boost of $CD34^+$ cells was administered.

Successful engraftment was observed in 7 of the 9 patients. The other 2 patients received a third transplant and 1 engrafted.[91]

In addition, preliminary data from the Tuebingen group also suggest that $CD34^+$ boosts may be clinically useful in patients with poor hematologic and immune reconstitution (i.e. $CD4^+ < 50$/uL) who also have infectious complications. In 4 such patients who received boosts, all exhibited a rapid immunological reconstitution accompanied by clinical improvement. One was noted to have a subsequent increase in the lymphocyte populations with simultaneous clearing of CMV infection; in the others there were no influence on lymphocyte recovery (R. Handgretinger, unpublished data). Other investigators have confirmed that stem cell boosts restore trilineage hematopoiesis and immune recovery in patients with malignant and non-malignant disorders after HSCT.[92,93]

2.10 Use of T-cell depleted HSCT grafts

Historically, the transplant program at St. Jude has used CD6/CD8 monoclonal antibodies with complement to deplete T lymphocytes from HAPLO and MUD bone marrow grafts. This result is a graft with a median of 5×10^5 T cells/kg. This antibody is no longer available from the manufacturer. Additionally, antibody-complement depletion methodologies do not adequately deplete peripheral blood stem cell grafts (which have a log more T cells than bone marrow) to achieve this target number of CD3 cells. Therefore, we have chosen OKT3 (Orthoclone, Muronomab-CD3), a murine monoclonal antibody comprising a purified IgG immunoglobulin that recognizes the CD3 antigen, as a new agent for T-cell depletion by negative selection. We feel that OKT3 is an ideal agent for T-cell depletion since it is an antibody with a narrow specificity and can be used with either peripheral blood or bone marrow grafts.[94]

Other investigators have used OKT3 for *ex vivo* T-cell depletion of mismatched family member bone marrow grafts. [48,94] Thirty-one patients were treated in this study with donor grafts containing a median of 1.06×10^8 nucleated cells/kg (range, $0.32\text{-}3.21 \times 10^8$ nucleated cells/kg) and 4.27×10^4 $CD3^+$ cells/kg (range, $0.61\text{-}75.5 \times 10^4$ $CD3^+$cells/kg). The median log T-cell depletion was 2.48 (range, 1.44-3.15). The study population also included an additional 35 patients receiving grafts T-cell depleted with T10B9 methodology. For the entire patient group, the estimated probability of engraftment was 0.96. Acute GVHD grade II-IV was observed in 24% and acute grade III-IV in 10%. The estimated probability of acute GVHD was not affected by degree of mismatch. The DFS at 3 years was 26% and the estimated probability of relapse at 3 years was 41%.

OKT3 methodology has been used in published studies for the *ex vivo* T-cell depletion of grafts from mismatched family members. Furthermore, Dr. Handgretinger has used this technology in Tuebingen in the mid-1990s. At that time, only the OKT3 antibody/microbead conjugates were available and he used his own magnetic system (not the CliniMACS device) for the depletion. In 3 patients, bone marrow from unrelated donors was depleted. The log depletion was higher than with PBSC, since the starting number of T cells in bone marrow is lower. A median log depletion of 4 was achieved. All 3 patients engrafted an ANC of $1000/mm^3$ at 13, 14, and 16 days. No acute or chronic GVHD was observed in the absence of GVHD prophylaxis. Two of the patients currently survive and are in remission; 1 patient has since relapsed.

A problem of the OKT3 depletion methodology is that these grafts contain more B-lymphocytes than those grafts obtained by $CD34^+$ positive selection. EBV-PTLPD, which is a potentially life-threatening complication, occurs when EBV, latent in B- cells, reactivates.

In this HIFLEX trial, we plan to T-cell deplete hematopoietic stem cell grafts from HAPLO donors using the CliniMACS system with the anti-CD3 antibody OKT3 labeled to magnetic microbeads. Mononuclear cells from mobilized peripheral blood (or alternatively bone marrow) from HAPLO donors will be incubated with the CliniMACS T cell CD3 microbeads. The depletion will be performed using the automated CliniMACS device with the 'Depletion' software. Our institution has performed 190 CD3 depletion processes on peripheral blood hematopoietic grafts, with a mean 4.15 log depletion of CD3+ cells. Post-depletion, the CD3+ content is 0.011%, CD19+ content is 10.84%, and the percentage CD34 recovery is 71.79. We have used this methodology regardless of graft source (bone marrow or PBSC).

To achieve the desired dose of HSC, it is anticipated that the HAPLO donors will undergo two sequential collections by apheresis. In the unusual situation of the target dose of T cells is reached from the first CD3+ depleted HSC product, the second product will be T-cell depleted using positive selection of CD34+ cells.

Several approaches have been developed to reduce the incidence of GVHD in the HAPLO donor setting. Because larger numbers of T cells (>3 x 10^6 $CD3^+$ cells/kg) has resulted in overall grade III-IV acute GVHD with significant morbidity and mortality[22] many investigators have utilized methods to remove T cells from grafts. These methods of T-cell depletion are based predominantly on negative selection strategies. These include physical methods such as T-cell specific lectins in association with sheep RBC rosetting or counterflow elutriation.[31-33,95] An alternate approach is the use of T-cell specific monoclonal antibodies in association with complement, immunotoxins or magnetic beads.[35-45,96] These approaches have resulted in an approximately 26-35% disease-free survival in high-risk leukemia and other high risk malignancies, with a 16-39% incidence of grade II-IV GVHD and a 5-10% non-engraftment rate.[20,21,38,39,42-46] However, despite the use of various preparative regimens in this group, there have been no demonstrable differences in outcome by the type of regimen employed. Overall, 30-45% of transplant recipients succumb to regimen-related toxicity with an additional 30%-40% dying from progressive disease or recurrence. [20,21,38,39,42-46]

Our institutional experience at St. Jude with CD6/CD8 T-cell depleted MUD-HSCT (HUD, MISMUD) demonstrate that outcomes following MUD-HSCT (including both 5/6 and 6/6 MUD as a single group) are similar to those from HLA-identical siblings.[18] These studies were based on the infusion of an allograft depleted of T cells for unrelated donors, primarily utilizing a CD6/CD8 monoclonal antibody/complement cocktail. We observed a significant reduction in the incidence of GVHD-related complications utilizing this approach, the incidence of acute grade II-IV being 22% with a 19% incidence of chronic GVHD. Coincidentally, our graft failure incidence remains quite low (less than 5%) in recipients of alternate donor grafts.[54] However, at our institution, HAPLO recipients continued to have inferior OS and DFS when compared to MUD recipients even when adjusted for the number of transplant recipients with high-risk disease, reflecting the historical observation that donor source is related to outcome.[55]

In our transplant clinical trial for patients with high-risk hematologic malignancy (HAPSCT), we enrolled 27 transplant recipient participants, distributed between two types of T-cell depletion: positive selection with the CD34 antibody and negative selection with OKT3. Of the 20 evaluable research participants receiving OKT3-depleted grafts, 12 have died (4 relapse and 8 regime-related toxicities). Six experienced acute GVHD, of the six 2 were overall grade III-IV. Chronic GVHD occurred in 4 participants (2 limited, 2 extensive). In addition, 2 participants developed PTLPD.

The Transplant Faculty have extensive experience with the proposed reduced intensity conditioning regimen as it has been used in our institutional REFSCT protocol, (Haploidentical Stem Cell Transplantation utilizing T-Cell Depletion as Therapy for Patients with Refractory Hematological Malignancies). From this protocol, we have gained substantial knowledge in respect to both the preparative regimen and the negative graft selection methodology that is, in part, employed in this study. Research participants received fludarabine 200 mg/m^2, melphalan 60-120 mg/m^2 depending on marrow aplasia, thiotepa 10 mg/kg, escalating and de-escalating doses of OKT3, and a negatively selected, CD3 depleted, mobilized stem cell graft from a HAPLO donor. GVHD prophylaxis consisted primarily of mycophenolate mofetil (MMF).

Conditioning and GVHD prophylaxis have been well tolerated by these REFSCT research participants, a population who has been heavily pre-treated prior to initiation of this transplant procedure. The 25 evaluable participants experienced a mean time to neutrophil engraftment (ANC greater than 500/mm^3) of 10.3 days (range 7-12 days) and platelet engraftment (greater than 20,000/mm^3 without transfusion support for the preceding 7 days) of 17.7 days (range 12 - 36 days). Two participants experienced non-engraftment with 1 having autologous reconstitution and the other requiring the infusion of their previous unrelated donor's stored stem cells. A total of 4 participants did not experience platelet engraftment prior to their deaths.

In this REFSCT trial, 14 transplant recipients died of primary/progressive disease. No unexpected AEs have been noted. Severe toxicities have been limited. Clinically significant NCI grade IV and/or fatal AEs that occurred included dilated cardiomyopathy, sepsis and multi-organ system failure. One recipient developed severe toxic epidermal necrolysis (TEN) at 91 days posttransplant. Four participants experienced clinically significant neuropathy. However, 3 of the 4 had either a history of neurological complications with prior therapy or central nervous system (CNS) disease involvement. The fourth patient was found to have brain infiltrates (consistent with fungal infection) believed to be the etiology of the neuropathy. This patient was also heavily sedated for a prolonged period of time, further confounding assessment.

Only 2 REFSCT research participants experienced overall grade III acute GVHD. Fourteen have experienced overall grade I-II acute GVHD; all responsive to therapy. Five participants have experienced extensive chronic GVHD (skin, oral, and/or gut) (institutional data, unpublished).

Since 2002, 27 children and young adults with hematologic malignancies underwent HAPLO HSCT on the HAPSCT trial (4 in 2002, 3 in 2003, 12 in 2004 and 8 in 2005). Since 2003, 25 research participants with refractory hematologic malignancies or requiring another allogeneic HSCT underwent HAPLO HSCT on the REFSCT trial (6 in

2003, 16 in 2004, 3 in 2005). These enrollment data demonstrate that accrual to these institutional trials has improved over time.

Our subsequent trial, HAPREF (Haploidentical Hematopoietic Stem Cell Transplantation Utilizing Partial T-Cell Depletion as Immunotherapy for Hematologic Malignancies) was then activated in 2006. In this trial, 5 of 9 research participants enrolled in the high-risk group (Group A) developed overall grade III-IV acute GVHD. At the same time point, 4 of the 8 research participants enrolled in the refractory Group B had also developed overall grade III-IV acute GVHD. These incidences of GVHD in both groups met the specified criterion for stopping accrual and reevaluation of the study. We concluded that the CD3+ dose used in this study was associated with excessive rates of acute GVHD.

For this reason, we altered the T cell ($CD3^+$) content of the stem cell graft, as this is strongly correlated with GVHD risk. The $CD3^+$ cell dose was reduced to 1.0×10^5 $CD3^+$/kg in the high-risk Group A. This specified $CD3^+$ dose is still higher than the median $CD3^+$ graft content in the prior HAPSCT (2×10^4 $CD3^+$/kg) and REFSCT (4×10^4 $CD3^+$/kg) clinical trials, theoretically allowing a more robust graft-versus-leukemia effect in this new trial.

2.11 Importance of donor selection.

There are several NK inhibitory receptors that recognize MHC class I molecules. Some of these receptors (KIRs) recognize specific determinants shared by certain class I alleles, and are clonally distributed among NK cells. Therefore, in the NK repertoire, some NK cells recognize and are blocked by specific class I alleles. Consequently, these NK cells can mount alloreactions against an allogeneic target if the target does not express the class I allele that blocks them. Ruggeri et al.[97] showed these reactions greatly improve outcomes of HAPLO transplantation. For poor risk AML research participants (mostly $\geq 3^{rd}$ complete remission or in relapse), the event free survival at 5 years was 5% if transplanted from HAPLO donors unable to mount NK alloreactions, but was 60% if transplanted from donors who do mount NK alloreactions. A recent study by the same group demonstrates that alloreactive NK cells may ablate leukemic cells, recipient T cells that cause rejection, and recipient dendritic cells that may trigger GVHD.[98]

Interest in the role of NK cells in HSCT developed from the observation of "hybrid resistance", in which lethally irradiated F_1 hybrid mice reject parental strain bone marrow yet tolerate other parental grafts.[99]Although the explanation of "hybrid resistance" is still debated, there is agreement that it is primarily mediated by NK cells. The authors stated that resting host-derived NK cells were capable of mediating resistance to both autologous and allogeneic marrow grafts; in addition, the transfer of activated donor NK cells mediated beneficial effects in allogeneic and syngeneic marrow transplantation. This observation and the generally recognized anti-tumor properties of NK cells led to exploratory studies to define the activity and manipulate the response of NK cells in transplant HSCT participants.

The Tuebingen group has reported the use of a low dose regimen of IL-2 posttransplant in leukemic research participants with high-risk for relapse. While the NK activity was low before starting the IL-2 treatment, a considerable increase could be measured during the low dose IL-2 treatment. After 7 weeks of therapy, the NK activity declined to starting levels prior to the IL-2 therapy.[100]

Since it has been shown that NK cells are able to lyse fresh leukemic target cells [101]and that allogeneic NK cells can control leukemia when given early posttransplant,[102] we are interested in the reconstitution of NK-cell subsets and their anti-leukemic cytotoxicity after HSCT. Since the cytotoxic function of NK cells is regulated via KIRs,[103] we are especially interested in the expression of these KIRs on the NK cells posttransplant. KIRs are receptors for HLA class I antigens and binding of these receptors to their corresponding ligands results in inhibition of cytotoxicity and other cell functions. [104] While the cDNA of a number of KIRs is known, only few can be identified via monoclonal antibodies and flow cytometry. Among the identifying receptors are CD94, CD158a, CD158b and DX9; we plan to analyze this surface expression on CD56+ NK cells periodically after HSCT.

From our institutional HAPSCT, MUDSCT and REFSCT protocols as well as from published data,[105]it is known that the first cells to reconstitute the immune system posttransplant are donor NK cells. Published studies from our institution show that NK-cell alloreactivity in haploidentical related donor-recipient pairs is associated with decreased relapse rates.[105,106] We propose to confirm the relationship between NK-cell activity and disease recurrence in a larger number of patients.

HAPLO donors will be selected preferentially for KIR ligand mismatches in the graft-versus-host direction. Selection for KIR mismatching is based on HLA typing as performed by serological and high-resolution molecular techniques. We will select the family donor with the most NK alloreactivity when possible.

2.12 Conditioning regimen considerations.

Patients undergoing HSCT require sufficient conditioning to prevent rejection, while allowing engraftment of donor hematopoietic cells to provide an antileukemic effect without increasing the risk of regimen-related toxicity.

The most commonly used conditioning regimens include combinations of busulfan and cyclophosphamide or cyclophosphamide and TBI. Our approach for this protocol will differ in that it prescribes a reduced regimen including melphalan, fludarabine, thiotepa and Campath-1H. TBI can increase the risk of acute organ toxicities and is also associated with an increased risk of second cancers and long-term complications, especially in children.[107-110] Avoidance of TBI may also decrease thymic damage and allow more rapid immune reconstitution posttransplant.[111]The use of busulfan is associated with risk for veno-occlusive disease of the liver (VOD).[112]Cyclophosphamide is an effective antileukemic drug that the majority of this patient population has already received in the past without benefit. Our institutional experience with HSCT using a non-TBI containing regimen for children and young adults with relapsed/refractory malignancy indicates that it permits engraftment with an acceptable risk of regimen related toxicity.[113]

We hypothesize that an immunoablative regimen will be better tolerated and will also provide an antileukemic effect. Data from Aversa et al and our institution[113,114] have demonstrated that fludarabine-based conditioning regimens can be used safely instead of TBI in HAPLO HSCT. In our institutional regimen of fludarabine, thiotepa, and melphalan, we reduced the dose of melphalan by 15% to decrease the gastrointestinal side effects. For those who had not recovered from the toxicities of prior therapy at the

time of HSCT, we may further reduce the melphalan dose by 50% to lower the risk of unacceptable toxicity and mortality.

In the REFSCT trial, using melphalan, fludarabine, and thiotepa, 2 of 25 enrolled transplant recipients developed peripheral neuropathy with white matter changes in the brain on MRI. One of the 2 progressed to a vegetative state and died of sepsis. Prior to HSCT, this participant had a history of severe vincristine-induced neuropathy, which had required him to be wheelchair bound. The second had persistent CNS leukemia and received intensive intrathecal therapy to induce remission prior to enrollment on the REFSCT protocol. This participant is steadily improving with supportive care and therapy and is an outpatient. Because no definitive etiology was found in either case and because fludarabine may cause neurologic abnormalities when administered at high doses, we have reduced the dose of fludarabine in this trial.

Lastly, regarding conditioning regimen considerations, T-cell reconstitution, as measured by CD3 quantitation in peripheral blood and based on T-cell receptor excision circles (TREC), was found to be more rapid in the more heavily pre-treated patient population on REFSCT trial than those treated on the HAPSCT trial.[115]

2.13 Use of Campath-1H in place of OKT3 in the conditioning regimen

We have been notified by Centocor Ortho Biotech, Inc that the manufacture and subsequent availability of OKT3 will cease in 2010. Therefore, an alternative agent is required for use in this protocol. The two agents which are most similar to OKT3 are ATG and Campath-1H. Campath-1H is chosen for Amendment 3.0 because ATG was found to be insufficient for engraftment of haploidentical graft and was associated with high risk of transplanted related mortality (TRM). Even in infants in whom megadoses of stem cells can be ensured, the rejection rate and TRM was still too high with ATG (1/8 engraftment failure rate and 3/8 TRM in our prior infant haploidentical transplant study – INFBMT). Furthermore, we have previously demonstrated that NK cells were highly susceptible to lysis by ATG, even more so than T cells.[116] Because NK cells are the key mediators of graft-versus-leukemia effects, ATG should be avoided in our transplant setting.

Campath-1H is a humanized monoclonal antibody with specificity for CD52.[117]Because Campath-1H is a recombinant monoclonal antibody with a consistent quality, there is not the lot to lot variability as is seen with ATG. Campath-1H has been shown to result in preferential immunodepletion of T cells, as CD52 is expressed on several leucocyte populations, but is most heavily expressed on T cells. CD34+ cells are largely unaffected by Campath-1H; thus making it an attractive candidate in bone marrow transplant settings. It has been used successfully in preparative regimens for MSD and MUD HCT, and in the doses used, Campath-1H has shown greater immunosuppressive effects in the form of decreased GVHD and delayed immune reconstitution.[117]

More recently Campath-1H has been incorporated successfully into haploidentical HCT.[118,119] Because Campath-1H has a long half life of approximately 5 days in children,[120]we will begin Campath-1H two weeks prior to graft infusion to maximize its effect on residual patient cells and engraftment, and minimize its effect on the graft and immune reconstitution.

2.14 Rationale for the measurement of Campath-1H pharmacokinetics

The pharmacokinetics (PK) of Campath-1H remain incompletely understood. Although Campath-1H was originally developed as a lymphocyte-depleting agent for HSCT, the majority of available PK data comes from adult chronic lymphocytic leukemia (CLL) patients.[121] Published PK data in children is limited, and involves significantly different dosing than this study.[120,122]

Combining study outcomes to date, the largest factor in clearance of Campath seems to be the burden of CD52.(Mould 2007) As such, Campath seems to have two phases of clearance: an initial rapid clearance when lymphoid tissues are being targeted and depleted, and a subsequent slow clearance when lymphoid tissues have been depleted. The half-life of Campath has been estimated to be as long as 15 to 21 days in this slow phase.[119] In T cell replete (unmanipulated) HSCT, Campath may remain at therapeutic levels as far out as 60 days post-HSCT.[125] Because the haploidentical grafts are heavily T cell depleted, the lymphoid burden (and consequently the CD52 burden) is predicted to be lower for the initial post-transplant period in our patients. Therefore, we propose to monitor Campath levels out to 16 weeks post-HSCT.

The PK of Campath-1H is of particular importance in HSCT as it can mediate depletion of both host and graft lymphocytes, thereby impacting both rejection and GvHD. Too much exposure to Campath can delay necessary immune reconstitution and increase infection risk, while too little may increase the risk of both rejection or GvHD.

2.15 Minimal residual disease (MRD)

Approximately 30 to 40 % of patients ultimately relapse after HSCT, despite the fact that the vast majority of patients are in clinical remission immediately before and after HSCT. Alternative approaches are needed to detect the residual leukemic cells that are below the limits of detection by standard morphologic examination. This is clinically important as studies confirm that, at least in CML, posttransplant relapse can be cured by interventions such as DLI, and the chance of cure is higher when the patients are still in MRD status. By using flow cytometry and polymerase chain reaction (PCR) amplification of antigen receptor genes in tandem, investigators at our institution have been able to conduct MRD studies in 80 consecutive ALL cases. Results of St. Jude institutional studies have shown that detection of MRD by immunologic techniques at any point in the treatment course is a powerful predictor of relapse in children with ALL.[123-127]However, other studies suggest that eradication of all acute leukemia cells may not be a prerequisite for cure.[123-127]

Similar to conventional-dose therapy, controversy also exists on the implication of MRD in the setting of HSCT. Unlike CML, there is a paucity of data on the natural history of AML and ALL patients who have MRD after HSCT, and how pre-transplant MRD levels influence posttransplant outcomes.[125,128,129]It is unclear whether they are also at greater risk of relapse, and whether further pharmacological or immunologic therapy indeed prolongs survival and increases cure rates. Thus, for those enrolled in this protocol who are unable to proceed to posttransplant immunomodulatory protocols, we will gather the MRD information together with hematopoietic chimerism in a descriptive manner to study the relationship between MRD and chimerism in this large cohort of patients. The

knowledge gained from this study should allow their future application to guide therapeutic interventions.

2.16 Rationale for decreasing CD3+ graft content

Due to the unacceptable rate of overall grade III-IV acute GVHD in the HAPREF trial (8/17, 47%) we have decreased the T cell content of the stem cell graft in this study, as we believe this was strongly correlated with GVHD risk. The $CD3^+$ dose of 1.5 x 10^5 $CD3^+$/kg used in the HAPREF study has been decreased to 1.0 x 10^5 $CD3^+$/kg. In 62 research participants who received a negative selected product in the 3 recent HAPLO HSCT protocols (HAPSCT [N = 20], REFSCT [N = 25], and HAPREF [n = 17]), one participant (3%) receiving a CD3 dose of < 1.5 x 10^5 CD3+ cells/kg experienced overall grade III-IV acute GVHD compared to 36% of those with a dose > 1.5 x 10^5 CD3+ cells/kg (p=0.0002, Table 1).

Table 1: Overall acute GVHD rate in relation to CD3 dose for recipients of negative selected donor products in HAPSCT, REFSCT, and HAPREF (N = 62)

(N = 62)	Acute GVHD	
	Overall grade 0 - II	Overall grade III – IV
Total CD3 x 10^5/kg	*N*	*N*
< 1.5 × 10^5/kg	29	1
≥1.5 × 10^5/kg	20	12
Total	49	13

Table 2: Overall acute GVHD rate in relation to participant age, donor product cell dose, and neutrophil (ANC) and platelet engraftment for recipients of negative selected donor products enrolled in HAPSCT, REFSCT and HAPREF (N = 62)

	Acute GVHD											
	Grade 0-2						Grade 3-4					
	N	Mean	Std	Median	Min	Max	N	Mean	Std	Median	Min	Max
Age at HSCT	49	11.45	5.65	11.95	2.83	26.55	13	12.89	6.08	15.20	2.67	20.71
Total CD34 cells 10^6/Kg	49	15.22	11.65	11.80	1.73	42.96	13	11.48	7.72	9.85	2.23	26.05
Total CD3 Cells x10^6/Kg	49	0.12	0.10	0.14	0.01	0.45	13	0.16	0.04	0.15	0.10	0.25
Days to ANC >=500	45	12.62	6.04	11.00	7.00	48.00	13	10.77	1.42	11.00	9.00	15.00
Days to Platelets >=20K	41	17.88	4.84	17.00	12.00	36.00	12	15.67	2.27	15.50	12.00	19.00
Time to Relapse	20	133.20	144.54	99.00	26.00	714.00	2	264.00	149.91	264.00	158.00	370.00

p = 0.0201

2.17 Chimerism analysis

Serial analysis of chimerism in peripheral blood and marrow of transplant recipients is extremely important to diagnose graft rejection, a significant problem after T-cell depleted grafts from mismatched donor-recipient pairs used for transplantation.[130,131] Because the initial signs and symptoms of graft rejection can be non-specific, the St. Jude Transplant Service presently measures peripheral blood chimerism weekly on all allogeneic transplant recipients. The definitive test for graft failure is chimerism performed on marrow or peripheral blood—reported as the percentage of cells of donor origin and the percentage of recipient origin. Presently chimerism is a DNA-based analysis using variable number of tandem repeats (VNTR) and the testing takes at least 48 to 72 hours to perform.

2.18 Predictive factors to identify risk for non-relapse morbidity and mortality

Non-relapse morbidity and mortality (NRM) is an important cause of transplant failure. NRM includes illness and death resulting from infection, acute and chronic GVHD, and organ failure not due to the underlying disease. Although HLA disparity is a major factor-identifying patients at risk for GVHD, other non-HLA factors have been demonstrated to be important in regimen-related toxicities. The inflammatory state of the patient before, during and shortly after conditioning may establish an environment in the transplant recipient that results in ongoing tissue damage and atypical antigen expression, leading to GVHD or other transplant toxicities.[132] The majority of studies of predictive factors for NRM have been performed in recipients of matched sibling or unrelated donor grafts; nearly all studies have been done in recipients of non-T-cell depleted grafts. For example, in animal studies, elevated tumor necrosis factor levels at day +7 posttransplant have been shown to contribute to the pro-inflammatory effects of GVHD.[132]Soluble interleukin-2 receptor has been shown in animal and human studies to be elevated during GVHD.[133]These two cytokines are important to study as there are established assays available and there are medications to block or inactivate each of these cytokines. Therefore, if elevated levels of these cytokines early posttransplant are demonstrated to be predictive of subsequent NRM, then early intervention may be indicated.

2.19 Rationale for present study

Haploidentical HSCT is complicated by the need for T-cell depletion of grafts due to the unacceptably high rates of severe acute GVHD when non-T-cell depleted products are used. However, this graft processing may lead to graft failure and delayed immune reconstitution. Our institutional experience suggests that a reduced intensity-conditioning regimen that omits total body irradiation and ATG may lead to a more rapid immune reconstitution and fewer infectious complications. Furthermore, a reduction in the CD3+ content of the graft will still allow engraftment, with more immunotherapeutic cells (monocytes, natural killer cells) being infused in the graft, potentially resulting in a graft-versus-malignancy effect and providing cells to fight infection, while potentially lowering the risk of GVHD observed when grafts of higher $CD3^+$ content are infused. Therefore, in this trial we will employ a reduced intensity-conditioning regimen of fludarabine, melphalan and thiotepa with a graft depleted of T cells using the anti-CD3 monoclonal antibody OKT3 on the CliniMACS system. Conditioning regimens that utilize this backbone, while avoiding TBI and ATG, enable a more rapid immune

reconstitution in our institutional HAPLO HSCT trials. In total, we aim to improve overall survival using a multimodal approach to reduce GVHD, enhance immune reconstitution, and reduce non-relapse mortality, ultimately establishing a platform on which to build posttransplant immunomodulatory strategies.

3.0 PROTOCOL ELIGIBILITY CRITERIA

3.1 Inclusion criteria (transplant recipient):

3.1.1 Patients less than or equal to 21 years old; may be greater than 21 years old if a current St. Jude patient or previously treated St. Jude patient within 3 years of completion of prior treatment.

3.1.2 Has one of the following diagnoses:

3.1.2.1 ALL high risk in second remission. Examples include: relapse on therapy, first remission duration of less than or equal to 30 months, or relapse within 12 months of completing therapy.

3.1.2.2 ALL in third or subsequent remission.

3.1.2.3 ALL high-risk in first remission. Examples include: induction failure, minimal residual disease greater than or equal to 1% marrow blasts by morphology after induction, persistent or recurrent cytogenetic or molecular evidence of disease during therapy requiring additional therapy after induction to achieve remission.

3.1.2.4 High-risk AML in first remission. Examples include: monosomy 7, M6, M7, t(6;9), FLT3-ITD, or patients who have greater than or equal to 25% blasts by morphology after induction or who do not achieve CR after 2 courses of therapy (includes myeloid sarcoma).

3.1.2.5 Relapsed or persistent AML (less than or equal to 25% blasts in marrow by morphology).

3.1.2.6 AML in second or subsequent morphologic remission (includes myeloid sarcoma).

3.1.2.7 CML in first chronic phase with detectable molecular or cytogenetic evidence of disease despite medical therapy; or CML with a history of accelerated or blast crisis, now in chronic phase; or unable to tolerate tyrosine kinase inhibitor therapy.

3.1.2.8 Juvenile myelomonocytic leukemia (JMML).

3.1.2.9 Myelodysplastic syndrome (MDS).

3.1.2.10 Therapy related (secondary) AML, ALL, or MDS.

3.1.2.11 Hodgkin lymphoma after failure of prior autologous HSCT or unsuitable for autologous HSCT.

3.1.2.12 Non-Hodgkin lymphoma (NHL) in CR2 or subsequent.

3.1.3 Has not received a prior allogeneic hematopoietic stem cell transplant.

3.1.4 Does not have a suitable HLA-matched sibling donor available for stem cell donation.

3.1.5 Does not have a suitable cord blood product or volunteer MUD available in the necessary time for stem cell donation (refer to subsection 4.1 for definition of "necessary time").

3.1.6 Has a suitable HLA partially matched family member available for stem cell donation.

3.1.7 Cardiac shortening fraction greater than or equal to 25%.

3.1.8 Creatinine clearance or glomerular filtration rate (GFR) greater than or equal to 40 ml/min/1.73 m^2.

3.1.9 Forced vital capacity (FVC) greater than or equal to 40% of predicted value or a pulse oximetry value of greater than or equal to 92% on room air.

3.1.10 Direct bilirubin less than or equal to 3 mg/dl.

3.1.11 Serum glutamic pyruvic transaminase (SGPT) less than 3 times the upper limit of normal for age.

3.1.12 Karnofsky or Lansky (age-dependent) performance score of greater than or equal to 50 (See Appendix A).

3.1.13 No known allergy to murine products or human anti-mouse antibody (HAMA) results within normal limits.

3.1.14 Not pregnant (confirmed by negative serum or urine pregnancy test within 14 days prior to enrollment).

3.1.15 Not breast feeding.

3.2 Inclusion criteria (stem cell donor):

3.2.1 Partially HLA matched family member.

3.2.2 At least 18 years of age.

3.2.3 Human immunodeficiency virus (HIV) negative.

3.2.4 Not pregnant (confirmed by negative serum or urine pregnancy test within 7 days prior to enrollment).

3.2.5 Not breast feeding.

3.3 Inclusion criteria (transplant recipient - stem cell boost)

3.3.1 Has experienced one of the following disorders posttransplant: graft failure, graft rejection, delayed hematopoietic and/or immune reconstitution. (Definitions provided in Sections 4.7 and 7.0 Evaluation Criteria).

3.4 Gender and minorities

According to institutional and NIH policy, this study will accession patients regardless of gender and ethnic background providing all inclusion/exclusion criteria have been met. Institutional experience confirms broad representation in this regard.

4.0 TREATMENT PLAN

GENERAL STUDY TREATMENT PLAN SCHEMA

Day	*Transplant Recipient Activities*	*Donor Participant Activities*
Day -14	Initiate conditioning regimen	
Day -5		Begin 6 days of HSC mobilization.
Day -1		Undergo day 1of apheresis.
Day 0	HSC infusion #1	Undergo day 2 of apheresis. Donor stem cells from day 1 of apheresis are processed using CliniMACS system.
Day +1	HSC infusion #2	Donor stem cells from day 2 of apheresis are processed using CliniMACS system.

4.1 Donor selection

Patients with either a suitable HLA-identical sibling MUD, or umbilical cord graft product, available in a timely manner will not be eligible for enrollment. The time period for consideration and identification of a suitable unrelated volunteer donor or cord blood graft will be defined as a minimum of 90 days from the time of obtaining the patient's HLA typing specimen until enrollment is requested. Patients who have no potential cord blood or volunteer unrelated donor identified in the registries on the initial search will proceed to HAPLO transplant providing all inclusion/exclusion criteria met. Those whose potential cord product or unrelated donors are completely typed and found not suitable and for whom no other suitable cord product or donors remain on the registries list may proceed to HAPLO transplant when indicated, even if prior to the 90 day timeframe.

This protocol will use HAPLO donors only. If more than one HAPLO family member donor is acceptable, then donor selection will be based on the largest KIR repertoire mismatch between donor and host, donor-recipient infectious disease markers, and donor health screen and examination.

Donor eligibility for stem cell collection will be determined through the use of the guidelines outlined in 21 CFR 1271 and the draft *Guidance for Industry: Eligibility Determination for Donors of Human Cells, Tissues, and Cellular and Tissue-Based Products (HCT/Ps)*. Potential donors will undergo a screening process which will include at least a physical examination, history and testing for relevant communicable diseases. The physical examinations to evaluate donor candidacy will be conducted by a non-transplant physician (St. Jude or non-St. Jude). A repeat physical exam is required prior to the boost stem cell collection process if there is no change in the health status of the donor and the initial physical exam was conducted greater than 180 days prior to the start of the boost stem cell collection process.

If a donor is determined to be ineligible, the donor is not automatically excluded. Part 21 CFR 1271.65 (b)(1)(i) allows for the 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). Standard donor testing will include for HIV-1 Ag, anti-HIV1, anti-HIV2, anti-HTLV-I, anti-HTLV-II, HBsAg, anti-HBc, anti-HCV, anti-CMV, and serologic tests for syphilis using FDA licensed test kits. Recipients or their legal guardians will be informed of the use of an ineligible donor.

4.2 Donor mobilization and donor stem cell graft collections

A mobilization regimen of granulocyte colony stimulating factor (G-CSF) will be used to obtain a PBSC product from the donor. The desired target goal will be ≥ 5 - 10 x 10^6 $CD34^+$ cells/kg, representing the total number infused in the transplant infusion irrespective of the number of days. This number of cells will be necessary to provide an adequate graft following the various *ex vivo* manipulations for prompt reconstitution.

The donor mobilization guidelines are as follows:

Days	Mobilization regimen
1, 2, 3, and 4	G-CSF 10 mcg/kg/day subcutaneous.
5 and 6	G-CSF 10 mcg/kg/day subcutaneous. Apheresis in St. Jude Donor Room.

It is anticipated that apheresis will be performed for a minimum of two days. Additional days of apheresis will be done at the transplant or apheresis physician's discretion. However, some donors may be able to provide the needed graft stem cell count after only one day of apheresis. If the donor is unwilling or unable to complete the mobilization process or apheresis procedure, a bone marrow product may be used. The bone marrow product will be processed using the same cell selection methodology on the CliniMACS device. Every effort will be made to infuse a fresh stem cell product; however, a frozen product may be infused when necessary.

All PBSC products will be collected as per American Association of Blood Banks (AABB) guidelines. Donors will be monitored during the time period of the mobilization and apheresis procedure with appropriate laboratory evaluation (see Appendix D).

4.3 Graft preparation

All cell processing will take place in the Human Applications Laboratory (HAL) in the Department of Therapeutics Production and Quality (TPQ) of St. Jude using established Standard Operating Procedures. The hematopoietic stem cell graft product will be T-cell depleted using the investigational CliniMACS device and CD3 Microbead reagent (OKT3 antibody conjugated iron-dextran beads) as directed by the manufacturer (Miltenyi Biotech). Briefly, hematopoietic progenitor cells collected by apheresis (HPC-A) from the mobilized donor will be initially assessed in the HAL and stored overnight at 4ºC. The next morning, the HPC-A product will be washed to remove platelets and adjusted to an appropriate cell concentration for incubation with the CliniMACS CD3 Microbead reagent in the manufacturer provided media. The cells will be washed to remove unbound microbeads. The cells will be applied to the CliniMACS device and the depletion will be performed using the "Depletion 2.1" software 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

stem cell (CD34+) and T cell content (CD3+) by flow cytometry. The HPC-A graft product will be infused fresh after completion of release testing and evaluation.

Under the unusual situation that the target number of CD3+ cells is reached on the first day of T-cell depletion, then the graft collected on subsequent days will be processed using CD34+ selection on the CliniMACS device. In rare circumstances, the product may be cryopreserved for later infusion to the recipient. The processing of the graft on the CliniMACS for CD34+ selection is similar to the processing steps for CD3+ depletion. In this case, the cells are positively selected with the "CD34 Enrichment" program on the CliniMACS after labeling with CD34 Microbead reagent per manufacturer's instructions.

Cell dose parameters for the *primary HSC* infusion donor graft are as follows. The cell doses noted are defined as the total CD3+ and CD34+ counts contained in the one donor stem cell product, irrespective of the number of infusions/days needed for the product to be administered (meaning that one final donor product is generally divided into 2 or 3 separate daily infusions. Thus, the doses below indicate the total donor product cell count).

- The target HSC dose is 10 x 10^6 CD34+ cells/kg. The minimum cell dose will be 2 x 10^6 CD34+ cells/kg with a maximum of 100 x 10^6 CD34+ cells/kg.
- The total T cell dose content in this primary infusion donor product will be $\leq$ 1.0 x 10^5 $CD3^+$ cells/kg and $\geq$ 0.01 x 10^5 CD3+ cells/kg.

Typically, the total graft will be provided with two sequential T-cell depleted HPC-A cell infusions. In rare situations, the minimum T cell dose may be achieved by the addition of T cells to the final graft product to reach the minimum dose of $\geq$ 0.01 x 10^5 CD3+ cells/kg.

4.4 Quality assurance of cellular product

Quality assurance for cell products is overseen by the TPQ Quality Assurance division, which authorizes release of all products. Only trained stem cell processors will process the cell products. Special training will be provided to all personnel operating the CliniMACS™ system. 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 standard operating procedures of the Human Applications Laboratory. All products will be tested for viability, sterility (culture and gram stain), and the presence of endotoxin. Culture and endotoxin results are not available before infusion of fresh 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 Human Applications Laboratory. The IRB and FDA will be notified, if at any time after infusion, cell product has been determined to be contaminated or endotoxin results exceed release limits.

4.5 EBV PTLPD prophylaxis

A dose of rituximab will be administered within approximately 24 hours prior to the stem cell transplant infusion.[134-136]This is provided as part of the pre-transplant preparative

regimen to prevent EBV PTLPD. This intervention has been chosen because of studies indicating that the EBV DNA level in the peripheral blood is suggestive of PTLPD. Rituximab has been demonstrated to be an effective therapy in PTLPD. The current standard clinical practice within the Division of Transplant is to administer rituximab when peripheral blood EBV DNA levels exceed 2000 copies/microgram. This is done regardless if the patient has concurrent clinical signs and symptoms in order to prevent an onset and/or progression of this PTLPD disorder. The medication will continue at the discretion of the treating physician as clinically indicated based on ongoing EBV copy levels and clinical assessment.

4.6 Preparative regimen –primary infusion

Recipients will receive the following standard conditioning regimen:

Day(s)	Treatment
Days -14 to -11	Campath-1H intravenous (dosing table below)
-9	Fludarabine 40mg/m^2 intravenous every 24 hrs (dose 1 of 5)
-8	Fludarabine 40mg/m^2 intravenous every 24 hrs (dose 2 of 5)
-7	Fludarabine 40mg/m^2 intravenous every 24 hrs (dose 3 of 5)
-6	Fludarabine 40mg/m^2 intravenous every 24 hrs (dose 4 of 5)
-5	Fludarabine 40mg/m2 intravenous every 24 hrs (dose 5 of 5)
-4	Thiotepa 5 mg/kg intravenous every 12 hrs
-3	Melphalan 60 mg/m2 intravenous (dose 1 of 2)
-2	Start MMF 600 mg/m2/dose intravenous twice a day
-2	Melphalan 60 mg/m2 intravenous (dose 2 of 2)
-1	Rituximab 375 mg/m2 intravenous
0	HSC infusion #1 on Day 0 HSC infusion #2 on Day +1 if subsequent infusion days may be needed
+6	Start daily G-CSF 5 mcg/kg/day subcutaneous or intravenous until ANC > 2,000/mm3 for 2 consecutive days and then as clinically indicated.

Notes:

- A reduced dose of fludarabine (i.e. 30 mg/m^2/day for 5 days) will be administered to participants with a history of significant neurologic disease, CNS-directed therapy or toxicity such as CNS irradiation, grade III-IV chemotherapy-induced neurotoxicity, and/or those receiving CNS radiation with this transplant.
- Participants in aplasia at the beginning of the conditioning regimen will receive a 50% reduction in melphalan dose as follows: 60 mg/m^2/day on day -3 and then day -2 no melphalan.
- MMF may be taken orally when recipient is able to tolerate solid or liquid formula.

- G-CSF may be administered for longer time periods if there are medical concerns regarding weak graft function or if there is an evident or possible (documented) concern for the existence of an active infection.
- Clinically indicated changes in dosing/days and supportive medications may be done for safety reasons when indicated such as in the case of allergic reactions, hypotensive reaction, etc. These changes will be reported as clinically indicated and in the continuing reviews where applicable.
- Although Campath-1H may be given subcutaneously for the first two doses, the final two doses must be given intravenously. Otherwise the dose outlined below in section 4.6.1 cannot be changed.

4.6.1 Preparative regimen – intravenous Campath-1H schedule

Day	Treatment	Participant less than 15 kg	Participant greater than or equal to 15 kg
-14	Campath -1H	1 mg	2 mg
-13	Campath -1H	10 mg	10mg/m^2
-12	Campath -1H	10 mg	15mg/m^2
-11	Campath -1H	10 mg	20mg/m^2

4.7 Additional stem cell administration ("stem cell boost")

Infusions of hematopoietic progenitor cells from the original donor may be performed for participants when clinically indicated for such conditions as graft failure, graft rejection, delayed hematopoietic recovery, or delayed immune reconstitution. Should the originally selected donor be unwilling or unable to donate cells following enrollment, an alternative haploidentical donor will be considered for the purposes of rescue. The boost will be processed on the CliniMACS^{TM} device using CD3 depletion methodology. The boost target doses will be $\geq 10 \times 10^6$ CD34+ cells/kg with a CD3+ cell/kg dose of $\leq 1.0 \times 10^5$ CD3+ cells/kg. These doses represent the total number of cells infused with the boost process, irrespective of the number of days the infusion.

Research participants for whom a graft processed with CD3+ depletion may not be clinically appropriate, such as in the case of active bronchiolitis obliterans organizing pneumonia or active acute GVHD, a graft from the original donor processed on the CliniMACS^{TM} device using CD34+ selection will be infused. The boost target dose for these participants is $>10 \times 10^6$ CD34+cells/kg with a CD3+ cell/kg dose of $\leq 5.0 \times 10^4$ CD3+ cells/kg.

In the event of graft failure or graft rejection, the following regimen will be provided prior to infusion of the stem cell boost product and a separate informed consent will be required. This regimen will be used regardless of the selection methodology used for the boost donor product.

4.7.1 Preparative regimen - stem cell boost:

Day	Regimen
-10	TLI (2Gy per fraction once a day) (TLI 1 of 4)
-9	TLI (2Gy per fraction once a day) (TLI 2 of 4)
-8	TLI (2Gy per fraction twice a day) (TLI 3 of 4 *and* 4 of 4)
-7	Fludarabine (40mg/m^2 intravenous every 24 hrs) (dose 1 of 3)
-6	Fludarabine (40mg/m^2 intravenous every 24 hrs) (dose 2 of 3)
-5	Fludarabine (40mg/m^2 intravenous every 24 hrs) (dose 3 of 3)
-4	Cyclophosphamide (50mg/kg intravenous once per day) (dose 1 of 3)
-3	Cyclophosphamide (50mg/kg intravenous once per day) (dose 2 of 3)
-3	Rabbit ATG (TEST DOSE 1mg/kg intravenous) (test dose 1 of 1)
-2	Cyclophosphamide (50mg/kg intravenous once per day) (dose 3 of 3)
-2	Rabbit ATG (3mg/kg intravenous) (standard dose 1 of 2)
-1	Rabbit ATG (3mg/kg intravenous) (standard dose 2 of 2)
0	HSC boost infusion (may be infused over more than 1 day)

Boost regimen related notes*:*

- Mesna will be administered intravenously for prevention of hemorrhagic cystitis from the medication cyclophosphamide. In general, mesna is administered at 12.5 mg/kg/dose prior to cyclophosphamide and at approximately 3, 6, and 9 hours post cyclophosphamide infusion. Mesna dose and administration schedule may vary based on physician recommendation.
- Rituximab 375 mg/m^2/dose will be administered intravenously within approximately 24 hours of the prior to boost infusion for PTLPD prophylaxis.

Notes: The following apply to all medication and infusion regimens noted in this protocol (primary /boost regimen/mobilization, etc):

- The stem cell infusion(s) may be delayed by approximately 24 hours in order to accommodate stem cell collection with the donor, the Blood Donor Center and/or Human Applications Laboratory as well as the recipient research participant clinical condition.
- The term “every” used in this regimen 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, dialysis or emergency diagnostic procedures.

- The term "day" does not refer to an absolute calendar day. It refers to an approximate, general 24 hour time period.
- Medication dosing may be modified for research recipients based upon actual body weight or adjusted ideal body weight when clinically indicated. Criteria for medication calculations based on body weight/body surface area can be found in any version of the St. Jude Formulary. Medication doses may be rounded to the nearest integer or to the nearest appropriate quantity when clinically or pharmaceutically indicated by the physician.

4.8 GVHD prophylaxis for the stem cell boost

We propose to T-cell deplete hematopoietic stem cell grafts from HAPLO donors using the CliniMACS system. Transplant recipients receiving a CD3+ depleted graft for an additional stem cell boost infusion will receive MMF prophylaxis beginning on day –2. Research participants who receive $\leq$ 2.5 x 10^4 $CD3^+$ cells/kg will have MMF discontinued on the last day of stem cell infusion. In general, for research participants without GVHD, MMF will be discontinued by approximately day + 60. Research participants who require an additional stem cell boost using a CD34+ positive selection methodology will not require administration of GVHD prophylactic medication.

4.9 Donor Lymphocyte Infusions (DLIs)/therapeutic cell, T cell infusions

DLIs may be administered from the original HSC donor for decreasing donor chimerism, serious viral infections or reactivations, or evidence of recurrent disease (including MRD or frank relapse). 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 approximately 300 ml whole blood. If the DLI is collected by leukapheresis, the amount to be processed would be approximately 2 total blood volumes.

Prior to DLI administration, the immunosuppression should be withdrawn and the recipient should have no active serious GVHD. The initial dose will be 2.5 x 10^4 $CD3^+$/kg. Subsequent doses may be escalated to 5 x 10^4 $CD3^+$/kg and then 1 x 10^5 $CD3^+$/kg if no moderate or severe GVHD occurs with the prior DLIs. No prophylactic immunosuppression will be administered with the DLI. The typical initial dose escalation for patients on this protocol is presented in the following table:

DLI DOSE AND SCHEDULE		
DLI	**Dose(10^4 $CD3^+$/kg)**	**Comments**
Initial Dose	2.5	Approximately 2-4 week intervals 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, an initial dose may be given for any chimerism less than 100% in a patient who is off immune suppression. The risk of

GvHD increases with increasing doses. In the last 5 years, no patient has developed moderate or severe GVHD after DLI dosed at 5 x 10^4 $CD3^+$/kg, though mild GVHD has occasionally occurred. Doses of 10 x 10^4 $CD3^+$/kg should be considered to carry a risk of severe GVHD. Therefore dose escalation to this level must incorporate the appropriate risk/benefit analysis by the treating transplant physician.

5.0 DRUG AND DEVICE INFORMATION

5.1 Fludarabine (Fludara)

Source and 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 25mg/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.

Toxicity: 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 of Administration: Intravenous.

5.2 Thiotepa (Thioplex® by Immunex) (TESPA, TSPA)

Source and 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.

Toxicity: 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 of Administration: Thiotepa will be administered as an intravenous infusion.

5.3 Melphalan (L-phenylalanine mustard, phenylalanine mustard, L-PAM, L-sarcolysin, Alkeran[â])

Source and 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% sodium chloride 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.

Toxicity: 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 bone marrow 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 of Administration: Intravenous

5.4 Mycophenolate mofetil (MMF, CellCept®)

Source and Pharmacology: Mycophenolate mofetil is hydrolyzed to mycophenolic acid (MPA), an immunosuppressive agent. MPA inhibits B and T-cell proliferation, T-cell synthesis, and antibody secretion by potent, noncompetitive reversible inhibition of inosine monophosphate dehydrogenase (IMPDH) in the purine biosynthesis pathway. Inhibition of IMPDH results in a depletion of guanosine triphosphate and deoxyguanosine triphosphate, important intermediates in the synthesis of lymphocyte DNA, RNA, proteins and glycoproteins. Oral formulations of mycophenolate mofetil are rapidly and extensively absorbed when given on an empty stomach. Food and aluminum and magnesium-containing antacids decrease absorption of MMF. MMF is rapidly

hydrolyzed to the active metabolite (MPA) after oral or intravenous administration. Free MPA is conjugated in the liver by glucuronyl transferase to inactive mycophenolic acid glucuronide (MPAG) that is excreted in the urine and feces. Time to peak plasma concentration is 0.8 – 1.3 hours, and the mean elimination half-life is 17.9 hours. Enterohepatic recirculation of MPA contributes to plasma concentrations. Administration of cholestyramine interrupts the enterohepatic recirculation and can decrease bioavailability by as much as 40%. Patients with renal insufficiency have increased plasma concentrations of MPA and MPAG. Acyclovir and ganciclovir may compete with MPAG for renal tubular secretion, resulting in increased plasma concentrations of both drugs.

Formulation and Stability: Mycophenolate mofetil is commercially available as 250 mg capsules, 500 mg tablets, 200 mg/ml powder for oral suspension, and 500 mg vials of powder for injection.

Supplier: Hoffmann La Roche, Inc.

Toxicity: Adverse events seen in patients taking mycophenolate mofetil include hypertension, hypotension, peripheral edema, leukopenia, anemia, thrombocytopenia, hypochromic anemia, leukocytosis, headache, insomnia, dizziness, tremor, anxiety, paresthesia, hyperglycemia, hypercholesterolemia, hypokalemia, diarrhea, hyperkalemia, hypophosphatemia, constipation, nausea, vomiting, anorexia, abdominal pain, dyspepsia, urinary burning or frequency, renal tubular necrosis, hematuria, increase serum creatinine and BUN, a variety of infections due to immunosuppression, rash, acne, ocular changes (cataracts, blepharitis, keratitis, glaucoma, and macular abnormalities) occasional leg cramps or pain, bone pain, myalgias, and hand cramps. Intravenous infusions have been reported to cause thrombosis and phlebitis. There have been occasional reports of gastrointestinal hemorrhage. High dose therapy with mycophenolate in adults with psoriasis has been associated with the following neoplasms: adenocarcinoma of the breast and colon, basal cell carcinoma, carcinoma of the gallbladder, histiocytic lymphoma, glioblastoma multiforme, and squamous cell carcinoma of the epiglottis.

Administration: Oral or intravenous.

5.5 Rituximab (Rituxan ™)

Source and Pharmacology: Rituximab is a murine /human chimeric monoclonal antibody. It is specific for the CD20 antigen located on B cells. It has been shown to mediate complement-dependent tumor cell lysis and antibody-dependent cellular cytotoxicity. Direct binding to the CD20 antigen is thought to play a role in inhibition of cell growth. Rituximab is administered intravenously. The mean serum half-life after a single intravenous dose of 375 mg/m2 is 59.8 hours (range 11.1-104.6 hours).

Formulation and Stability: Rituximab is available as 100 mg/10 ml single-use and 500 mg/50 ml single-use vials. Each vial also contains sodium chloride 9 mg/ml, sodium citrate 7.35 mg/ml, polysorbate 80 0.7 mg/ml and water for injection. Rituximab for injection concentration must be diluted with 5% Dextrose or 0.9% NaCl prior to administration. After dilution, unused drug is stable for 24 hours when refrigerated (2-8 degrees Celsius) and 12 hours at room temperature. Vials should be protected from direct sunlight.

Supplier: Commercially available.

Toxicity: Hypersensitivity reactions may occur; therefore, premedication with acetaminophen and diphenhydramine should be considered before each infusion. The most common toxicities are infusion related and may include chills, fever, headache, nausea, vomiting, angioedema (13%), hypotension (10%), bronchospasm (8%), and arrhythmias. Other possible adverse reactions include thrombocytopenia, myalgias, arthralgias, asthenia, and throat irritation.

Route of Administration: Intravenous. Do not administer as an intravenous push or bolus.

5.6 Alemtuzumab (Campath-1H, Campath®)

Source and Pharmacology: Alemtuzumab is an unconjugated, humanized monoclonal antibody against the CD52 antigen, which is expressed on the surface of B-lymphocytes, T-lymphocytes, monocytes, and platelets but not hematopoietic stem cells. It has preferential effects in the blood and bone marrow as opposed to the spleen or lymph nodes. Alemtuzumab is associated with profound T-lymphocyte depletion. It mediates the lysis of lymphocytes via complement and antibody dependent cell mediated cytotoxicity mechanisms. Alemtuzumab is associated with the release of tumor necrosis factor (TNF), interleukin-6, and interferon gamma.

Formulation and Stability: Alemtuzumab is available as a clear, colorless, isotonic, sterile phosphate buffered saline solution at 10 mg/ml in 3 ml ampules. It is stable at least 12 months if refrigerated and protected from light. After dilution in NS or 5% dextrose, alemtuzumab is physically and chemically stable at room temperature for up to 24 hours. The manufacturer recommends use within 8 hours of preparation due to absence of a preservative. At concentrations above 20 mcg/ml, there was no detectable loss of drug associated with IV administration sets. A sterile, low-protein binding, non-fiber releasing 5 micron filter should be use when removing the medication from the ampule. The medication ampule should not be shaken. The admixture should be mixed by gently inverting the bag containing the final solution.

Toxicity: Infusion related reactions occur in most patients, and commonly include rigors, fever, headache, nausea, vomiting, diarrhea, rash, pruritis, dyspnea and hypotension. Also reported are chills, abdominal and back pain, bronchospasm, angioedema, and tachyarrhythmias. These reactions are most common during the first week of treatment, and usually improve with subsequent doses. Other expected adverse events are anemia, neutropenia, thrombocytopenia, prolonged and profound lymphopenia, and bacterial and opportunistic infections. Also reported are fatigue, hypertension, angina and myocardial infarction, peripheral vasoconstriction, anorexia, constipation, dyspepsia, liver function abnormality, DIC, hemolysis, eosinophil disorder, bleeding (GI, gum, ecchymosis), myalgia, arthritis, bone pain, hypotonia, tremor, tumor lysis syndrome, acidosis, dizziness, confusion, somnolence, peripheral neuropathy, cerebral hemorrhage, speech disorder, paresthesia, syncope, depression, aphasia, cough, pleural effusion, pulmonary edema, interstitial pneumonitis, facial flushing, diaphoresis, urticaria, oliguria, polyuria, urinary retention, urinary tract infection, impotence, tinnitus, and injection site reactions if given SC.

Supplier: Campath® is produced by Ilex Oncology Services and Millennium Pharmaceuticals and distributed by Berlex Laboratories. It has been designated an orphan drug for the treatment of chronic lymphocytic leukemia and is commercially available.

Dosage and Route of Administration: Alemtuzumab is associated with infusion related reactions, especially with the first dose. The severity of these reactions can be decreased by premedication with diphenhydramine and acetaminophen 30 minutes prior to infusion, and by starting with a low dose and gradually increasing to the target dose. The dose should be mixed in NS or 5% dextrose at a concentration greater than 20 mcg/ml and administered over 2 hours through a 0.22 micron filter. Alemtuzumab can be administered subcutaneously.

5.7 Cyclophosphamide (Cytoxan)

Source and 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

Toxicity: Dose limiting toxicities of cyclophosphamide includes bone marrow 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 cyclo-phosphamide 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 of Administration: intravenous infusion

5.8 Mesna (Mesnex)

Source and 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

Toxicity: 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 cyclo-phosphamide dose. It is normally ordered to be given intravenously prior to and again at 3, 6 and 9 hours following each dose of cyclophosphamide.

5.9 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 β2 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. Infusions should be prepared immediately prior to administration.

Supplier: Commercially available.

Toxicities: Frequently reported events 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. 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 of administration: Intravenous. Test doses may be infused over 1 to 2 hours. The first full dose should be infused over a minimum of 6 hours. If tolerated, subsequent doses may be infused over 4 hours.

5.10 G-CSF (Filgrastim, Neupogen®)

Source and 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.

Toxicity: 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 of administration: Intravenous or subcutaneous.

5.11 CliniMACS™ System using CD3+ depletion [137]

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 OKT3 antibody used in this system is highly specific for CD3+ cells. High-gradient MACS technology has been shown to achieve rapid and highly specific depletion of large numbers of CD3+ cells from bone marrow, cord blood, and normal peripheral blood mononuclear cells.

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

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 CD3+ cells. The CliniMACS device is not licensed by the FDA and therefore is investigational.

5.12 CliniMACS™ System for CD34+ positive selection [138]

The CliniMACS device has separate programs that allow cell selection procedures optimized for either a depletion (e.g. CD3 depletion) or a selection of a target cell population (e.g. CD34+ 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. In this protocol, if required, CD34+ hematopoietic stem cells from the original donor will be positively selected with the CliniMACS and used as the graft.

6.0 REQUIRED OBSERVATIONS/EVALUATIONS

6.1 Pre/peri/post-transplant evaluations

All pre/peri/posttransplant and long-term follow-up 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: http://home.web.stjude.org/bone_marrow/clinicalHome.shtml.

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 posttransplant in the Department of BMTCT outpatient clinic. For the purpose of this study, research participants will be followed to year 5 post-transplantation (refer to Appendix D for long-term follow-up requirements). At the 1 year post-transplantation time point, all study participants will be eligible for enrollment in the institutional long-term follow-up protocol for children and young adults who have received stem cell transplantation at St. Jude Children's Research Hospital (BMTFU protocol).

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. However, for research participants who have less than 95% donor chimerism at/about day +21 posttransplant, a repeat bone marrow study (to include chimerism) may be performed approximately 1 week after the initial procedure, at the discretion of the treating physician. 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) $\geq 500/mm^3$. 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 7 days. Patients requiring platelet transfusions to maintain platelet counts above the previously defined values will not be evaluable for these outcomes.

Chimerism studies will be performed by VNTR methodology performed in Molecular Pathology with a time to result of approximately 48 hours. 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 2 weeks to confirm this initial decline. In addition, chimerism analysis will be performed in subsets of lymphocytes, granulocytes, and monocytes for research participants with increasing host chimerism until the research participant attains $\geq$ 95% donor chimerism. Chimerism studies will be performed in the St. Jude Department of Pathology using standard DNA techniques [i.e. VNTR (variable number tandem repeat)] and/or FISH (fluorescent in-situ hybridization) analysis. Chimerism studies are reported in the database as donor percentages.

6.4 Evaluation for immune reconstitution

Research participants will have immune reconstitution studies of lymphocyte subsets (i.e. cells, B cells, and NK cells). These studies may be performed more often than what is outlined in Appendix D depending on the clinical status of the research participant.

Immune analysis as described (Mnemonic listed in 6.4.1 through 6.4.4) will be 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 VBETA/TREC Research: Thymic output and T cell repertoire.

6.4.3 Lymphocyte Phenotypes Research: T cell and NK cell number and function.

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

6.4.5 Soluble interleukin-2 receptor (sIL-2r) and tumor necrosis factor (TNF-alpha, TNFR1): Testing will be performed weekly starting on day -7 and then weekly until on or about day +100 posttransplant.

6.5 General viral surveillance

Serial PCR testing for CMV, adenovirus, and EBV will be done weekly to day 100 and then on an as needed basis. In research participants with progressive or active infection, samples may be obtained more frequently (St. Jude test mnemonic BMTPCR).

6.6 Evaluations for Campath-1H pharmacokinetics (optional)

Campath-1H pharmacokinetics will be measured on up to 15 recipients. The participants will be divided into 3 cohorts, with up to 5 recipients in each: 1) less than 15kg; 2) greater than 15kg and less than $1m^2$; 3) greater than $1m^2$. The following time points will be used for each patient: Day -10, Day -8, Day -3 before transplant, and then at weeks 1, 2, 4, 8, and 16 after transplant. Approximately 2mL of peripheral blood will be drawn at each time point. The samples will be stored frozen and will be shipped in dry ice, in batches, every 6 months, to Genzyme for analysis.

The responsibilities and procedures for PK sample collection, submission, and analysis are outlined in APPENDIX F.

6.7 Minimal residual disease (MRD) evaluation

MRD assays in peripheral blood and/or bone marrow by immunologic and molecular methods will only be performed for those research participants who have had this test performed during prior therapy for their disease at St. Jude or for those who have samples of diseased marrow available to identify a leukemic marker for MRD testing. Bone marrow and/or peripheral blood will be obtained from the research participants at specific time points (refer to Appendix D for schedule of MRD testing). MRD assays may be performed more frequently in research participants with increasing host chimerism. We will apply immunologic and molecular methods, as previously described.[123,124] Tests will be performed in the appropriate St. Jude laboratories.

6.8 Research tests on haploidentical donor (optional)

Donors will be offered the option for participation in research studies of immune reconstitution of T cells, B cells, and NK cells. 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:

6.10.1 Lymphocyte subsets study: Flow cytometry enumeration.

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

6.10.3 Lymphocyte Phenotypes Research: T cell and NK cell number and function.

6.10.4 Soluble interleukin-2 receptor (sIL-2r) and tumor necrosis factor (TNF-alpha, TNFR1)

7.0 EVALUATION CRITERIA

7.1 Adverse event (AE) monitoring for on-study research participants will be assessed using the NCI Common Toxicity Criteria Version 3.0. The specific criteria for AE monitoring are noted Section 9.0 and in Appendix C.

7.2 GVHD scoring (acute and chronic) will be evaluated and graded using the criteria found in Appendix B of this protocol.

7.3 Performance status will be assessed by Karnofsky/Lansky Performance Scores (age-dependent) (see Appendix A).

7.4 Hematologic recovery posttransplant will be determined using the engraftment criteria as follows: (1) neutrophil engraftment will be defined as the first of 3 consecutive days of an ANC $\geq 500/mm^3$ with evidence of donor cell engraftment; (2) platelet engraftment will be defined as platelet count $\geq 50{,}000/mm^3$ with no platelet transfusions in the preceding 7 days.

7.5 Primary graft failure will be defined as an ANC never meeting or exceeding $500/mm^3$ for 3 consecutive days and no evidence (<10%) of donor chimerism by day +21 posttransplant.

7.6 Secondary graft failure or graft rejection will be defined as no evidence (<10%) of donor chimerism in research participants with prior neutrophil engraftment.

7.7 Mixed hematopoietic chimerism will be defined as between 10% and 95% donor chimerism in the absence of immunosuppressive therapy.

8.0 OFF-STUDY AND OFF-THERAPY CRITERIA

8.1 Transplant recipient research participants will remain on-study until one of the following occurs (i.e. off-study criteria):

8.1.1 Withdrawal from protocol. Research participants or legal guardian(s) may withdraw consent to participate in this study at any time. Physician may withdraw participant if at any time the treatment is deemed unsafe or not in the participant's best interest.

8.1.2 Death.

8.1.3 Donor unable to provide the stem cells/stem cell doses required for intended recipient to undergo primary stem cell transplant.

8.1.4 Requires an additional transplant procedure using a different allogeneic donor (meaning a donor other than the HAPLO donor used for this protocol procedure).

8.1.5 Unable to be contacted and/or effectively monitored by the principal investigator (PI) and/or designees for follow-up (lost to follow-up).

8.1.6 Five years post primary stem cell infusion (i.e. has completed the year +5 post primary transplant evaluation).

8.2 Transplant recipient research participants will remain on-study but considered off-therapy if one of the following occurs (i.e. off-therapy criteria):

8.2.1 Requires additional chemotherapy for recurrent disease.

8.2.2 Experiences graft failure or rejection despite receiving one stem cell boost infusion.

8.2.3 Experiences graft failure or rejection despite receiving more than two donor lymphocyte infusions within the first 100 days post primary transplant infusion.

8.2.4 Noncompliance with protocol medications/administrations and/or required follow-up evaluations as judged by the PI.

8.2.5 Pregnancy.

8.2.6 Donor has been removed from study *after* the primary HSCT has been administered (criteria for donor removal for study – see below) and the recipient requires a stem cell boost. Meaning donor unable to provide the additional cells required for the boost infusion if needed.

Notes:

- Off-therapy transplant recipient research participants will be followed for disease status, survival status (cause of death /date of last follow-up) and GVHD only until the time an off- study criterion is met.

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

8.3.1 Donor research participant withdrawal. Donor research participants may withdraw their consent to participate at any time.

8.3.2 Off-study date will be determined once the PI has established that the transplant recipient does/will not require the primary transplant infusion, or an additional infusion for the purpose of this protocol.

8.3.3 Seven days post final cell collection procedure.

8.3.4 Day of transplant recipient death.

8.3.5 Pregnancy.

8.3.6 Development of a health disorder, including a clinically significant risk for/positive testing for communicable disease, which in the opinion of the PI

would render the donor ineligible to serve (or continue to serve) as a stem cell donor.

8.3.7 Death.

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

8.3.9 Noncompliance with protocol mobilization medications and/or apheresis procedure(s) and/or required testing as judged by the PI.

9.0 REPORTING CRITERIA

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, by phone: (901) 595-4359, cell: (901) 336-2894, fax: (901) 595-4361, or e-mail: hsp-1@stjude.org. A reportable event entry into TRACKS 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 exceptions:

- 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.
- Any episodes of overall grade III or IV acute GVHD in participants will be reported to the IRB as soon as possible but no more than within 10 business days of the PI's confirmation of the diagnosis/grade of the event.
- Clinical diagnosis of PTLPD will be reported to the IRB as soon as possible but no more than within 10 days of the PI's determination of the disorder.

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 B, and 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 mobilization with growth factors

begins, until 7-days post last day of the final apheresis procedure. If the transplant recipient requires a second HSC 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 requirements to the St. Jude Institutional Biosafety Committee (IBC)

Continuing review reports will be sent to the St. Jude IBC on at least an annual basis using the most current version of the continuing review form found on the Institutional Biosafety Committee website. The safety reports, sent to the IRB for both the donors and stem cell recipients, will be simultaneously forwarded to the St. Jude IBC. Therefore, reporting for safety events to this committee will be according to the same timelines as reporting to the IRB. As per the direction of the IBC, only those protocol revision and

amendments that are 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 requirements to the FDA

Any unexpected fatal or unexpected life-threatening event judged by the PI to possibly be due to the study treatment, will be reported to the FDA by telephone or fax as soon as possible but no later than 7 calendar days after notification of the event and followed by a written safety report as complete as possible within 8 additional calendar days (i.e. full report 15 calendar days total after notification of event).

Unexpected, non-fatal and non-life-threatening SAEs, which occur in on-study participants during the time periods specified in Section 9.1 that are considered due to or possibly due to the investigational agent, will be reported to the FDA by written safety report as soon as possible but no later than 15 calendar days of the notification of the occurrence of the event. Expected SAEs, even unexpected fatal SAEs, considered by the PI to be not related to the study, will be reported to the FDA in the annual review report. A summary of the safety of DLIs will also be provided to the FDA in the annual review report.

9.4 Summary of reporting mechanism by the St. Jude Office of Regulatory Affairs

Copies of all correspondence to the St. Jude IRB, including SAE reports, are provided to the St. Jude Office of Regulatory Affairs. 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 AEs will be filed with the St. Jude IRB, IBC and FDA at least annually.

9.6 Reporting to the St. Jude Data Safety Monitoring Board

This study has been referred to the St. Jude Data and Safety Monitoring Board (DSMB) for regular monitoring. The DSMB is charged with advising the Director and other senior leaders of St. Jude on the safety of clinical protocols being conducted by St. Jude investigators and on their continuing scientific validity. DSMB monitoring and review for this study will be conducted in accordance with the NCI guidance for DSMBs on an approximate semiannual basis. The St. Jude DSMB is responsible for ongoing review of the protocol and related information such as enrollment, issues related to participant safety (specifically toxicities and the risk:benefit ratio of the trial), interim analyses, and the overall study conduct necessary to accomplish the primary protocol objectives. This includes evaluation of the accrual rate, adherence to the study design, outcome measures, and review of protocol related primary outcome data. The PI will meet with DSMB during the semiannual visits to review the information submitted and discuss the status of the protocol. The DSMB may recommend that the trial be modified, suspended to accrual, and/or stopped based on their review.

9.7 Data submission to Miltenyi Biotec

Clinical and safety related data will be provided to Miltenyi Biotec, the manufacturer of the CliniMACS system. Data will include but is not limited to the transplant research participant's age and diagnosis, donor product(s) related information including HAPLO donor type (e.g. parent, sibling), the stem cell mobilization, selection, and infusion procedure. Outcome data including lymphohematopoietic reconstitution, immunological response, disease response and transplant complications will be shared with Miltenyi Biotec. Representatives from Miltenyi Biotec will be able to review the research participant's (donor and transplant research recipient) laboratory and medical record for data verification purposes. Copies of all reports to the governing regulatory bodies will also be accessible to Miltenyi Biotec upon request.

In the event that the protocol is placed on a clinical hold by the PI or governing regulatory authorities (IRB and/or FDA), representatives from Miltenyi Biotec will be notified as soon as possible.

9.8 Reporting to bone marrow transplant registry

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). The CIBMTR is responsible for the collection and maintenance of a standardized database of all autologous and allogeneic (related and unrelated donor) transplants performed in the United States.

Data sent to the CIBMTR will include but is not limited to the transplant recipient's date of birth, country/state of current residence, diagnosis, previous medical therapy including prior chemotherapy regimens and doses. Other information will include the relationship of the donor to the transplant recipient (i.e. parent, sibling), HLA type, and donor product(s) related information including the stem cell mobilization, selection and infusion procedure. Outcome data including short- and long-term lymphohematopoietic reconstitution, immunological response, disease response and transplant complications will be shared with the CIBMTR. Donor participant information will also be included and will be more limited to information such as age in years, relationship to recipient, and infectious disease testing. Information for both donor and recipient is submitted using a unique research participant number.

10.0 STATISTICAL CONSIDERATIONS

This primary focus of this protocol is to improve the outcome for the patients receiving CD3-depleted allogeneic stem cell graft obtained from HAPLO family member donors to treat patients who lack a matched sibling donor or a MUD. The approach adopted in this protocol is derived from the experiences gained from three previous institutional protocols HAPSCT, REFSCT and HAPREF.

In HAPSCT research participants with high-risk risk hematologic malignancies received a conditioning regimen containing TBI, ATG, cyclophosphamide and thiotepa. Cyclosporine was used for GVHD prophylaxis. On the other hand, REFSCT enrolled research participants

with refractory disease or who required second (or subsequent) allogeneic HSCT to receive a reduced intensity conditioning regimen of melphalan, fludarabine and thiotepa. MMF was used as the GVHD prophylaxis. Following the completion of both trials and the analysis of data revealed that the research participants treated on REFSCT has a more rapid immune reconstitution, a lower rate of viral reactivation and a lower regimen-related toxicity as compared to those on HAPSCT. Both trials had low rates of overall grade III-IV acute GVHD.

Because of the promising results of REFSCT, a trial (HAPREF) was initiated to treat both groups of research participants. These participants with hematological malignancies were treated on two strata: Arm A-high-risk ("like HAPSCT"), and Arm B-refractory and/or second (or subsequent) transplant ("like REFSCT"). All participants received a non-TBI based less intensive conditioning regimen, the same GVHD prophylaxis but a higher CD3+ cell dose in the graft (the primary transplant infusion donor product) with the expectation that it would significantly reduce the relapse rate. However, most likely, this higher T cell dose resulted in significantly higher grade III-IV acute GVHD in both strata and the protocol was closed after reaching the stopping rules.

Thus, in proposing a new trial for the high-risk patient population (i.e. those treated on HAPSCT and those in Arm A of the HAPREF protocol), a major change from HAPREF protocol has been made to reduce the total T cell dose in this primary donor graft product from 1.5 x 10^5 to a total dose of ≤ 1.0 x 10^5 cells/kg. The justification for the choice of this T cell dose is based on the analysis of previous protocols. We analyzed the data of the recipients of negative selected donor products in the HAPSCT trial (N = 20), all participants in the REFSCT (N = 25) and HAPREF studies (N = 17 total for both arms) to better understand the relationship between total T cell dose in the primary donor product infused and incidence of acute GVHD. The findings are reported in the following Table 3:

Table 3. Overall acute GVHD, relapse, graft failure/boost, and DLIs for recipients of negative selected donor products in HAPSCT, REFSCT, and HAPREF (N = 62)

	Overall Grade Acute GVHD		Relapse?		Graft Failure		Receive Boost within 100 days		Receive DLI within 100 days?		
	0-II	III-IV	No	Yes	No	Yes	No	Yes	No	Yes	*Total*
Primary Transplant Infusion CD3 cells	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	***N***
<=1.0 x 10^5 cells/kg	20	1	14	7	19	2	19	2	16	5	21
> 1.0 x 10^5 cells/kg	29	12	27	14	39	2	38	3	37	4	41
Total	49	13	41	21	58	4	57	5	53	9	62

*Overall acute GVHD: p-value = 0.0445**;** Relapse: p-value =1.0; Graft failure: p-value=0.5987; DLIs: p-value = 0.25*

It is seen from the above table (Table 3) that 1 of the 21 (5%) participants who received a total T- cell dose of ≤1.0 x 10^5 developed grade III-IV overall acute GVHD, whereas 12 of the 41 (29%) who received the total T cell dose of >1.0 x 10^5 had grade III-IV. This was

significant with an exact p-value, based on binomial distribution, of .0445. However, a lower rate of occurrence of grade III-IV acute GVHD would be suggestive of a higher relapse rate and it was seen that relapse rate was 7/21 (33%) and 14/41(34%) in the two T cells groups. The exact p-value of 1.0 suggests that there is no significant difference in the incidence of relapse between those who received a lower T cell dose and those who received a higher T cell dose. In addition, the p-value for the incidence of graft failure and the need for DLIs for the two groups do not appear to be significantly different with p-values of 0.5987 and 0.25, respectively. Thus, in this new protocol with the modifications suggested (for details see Section 4.0) it is expected that the overall grade III-IV acute GVHD rate would be significantly reduced. Further, with the use of non-TBI based and less myeloablative conditioning, we expect to reduce regimen-related toxicity, have faster immune reconstitution and also have a lower infection rate. Thus, with this approach we expect to significantly improve the outcome in terms of event free survival (EFS) in transplant recipients in this trial.

A similar analysis for the research participants with these high-risk hematologic malignancies treated in Arm A of HAPREF (N = 9) and those who received negatively depleted graft on the HAPSCT (N = 20) suggested similar findings as depicted in the following table:

Table 4. Acute GVHD, relapse, graft failure and additional cell infusion(s) by total T cell dose in HAPREF Arm A and recipients of negative selected products in HAPSCT (N = 29)

	Overall Grade Acute GVHD		Relapse?		Graft Failure?		Receive boost within 100 days?		Receive DLI within 100 days?		
	0-II	III-IV	No	Yes	No	Yes	No	Yes	No	Yes	Total
Primary Transplant Infusion CD3 cells	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*	*N*
<=1.0 x 10^5 cells/kg	10	1	9	2	11	0	9	2	7	2	11
> 1.0 x 10^5 cells/kg	12	6	14	4	17	1	15	3	17	3	18
Total	22	7	23	6	28	1	24	5	24	5	29

Overall acute GVHD: p-value =0.2021; Relapse: p-value = 1.0; Graft failure: p-value=1.0; DLIs: p-value = 1.0

It is seen from the above table that 1 out of 11 patients (9%) who received a total T cell dose of ≤1.0 x 10^5 cells/kg developed grade III-IV overall acute GVHD, whereas 6 of the 18 (33%) who received the total T cell dose of > 1.0 x 10^5 cells/kg had grade III-IV. This was not statistically significant with an exact p-value, based on binomial distribution, of 0.2021 but was suggestive of the fact that patients who received a higher T cell dose are likely to have more GVHD. Also, the relapse rate was 2/11 (18%) and 4/18 (22%) in the two T cells groups. There does not appear to any statistically significant difference in the incidence of relapse, incidence of graft failure or need for DLIs with exact p-values of 1.0 for all 3 comparisons. Because of the small sample sizes the results should be interpreted with

caution. However, from the analyses presented above it is clear that only 1 participant who received a total T cell dose of ≤ 1 x 10^5 developed this acute GVHD and that did not seem to affect the relapse rate or graft failure rate. Thus, we expect that administering a total T cell dose of ≤1 x 10^5 cells/kg should be relatively safe and should help in improving the outcome of the research participants on this protocol.

Further, focusing only on these 29 participants with high-risk hematologic malignancies who received negatively depleted grafts on HAPSCT and Arm A of HAPREF, it is seen that one-year EFS estimates are 40.0%±10.3% and 55.6%±16.6%, respectively. The Kaplan-Meier plots along with the survival estimates are provided in the tables below (Tables 5 and Table 6):

Table 5. Event-free survival in HAPSCT negative selection recipients and HAPREF Arm A participants (N = 29 total)

Event-free survival in HAPSCT negative selection and HAPREF Arm A participants

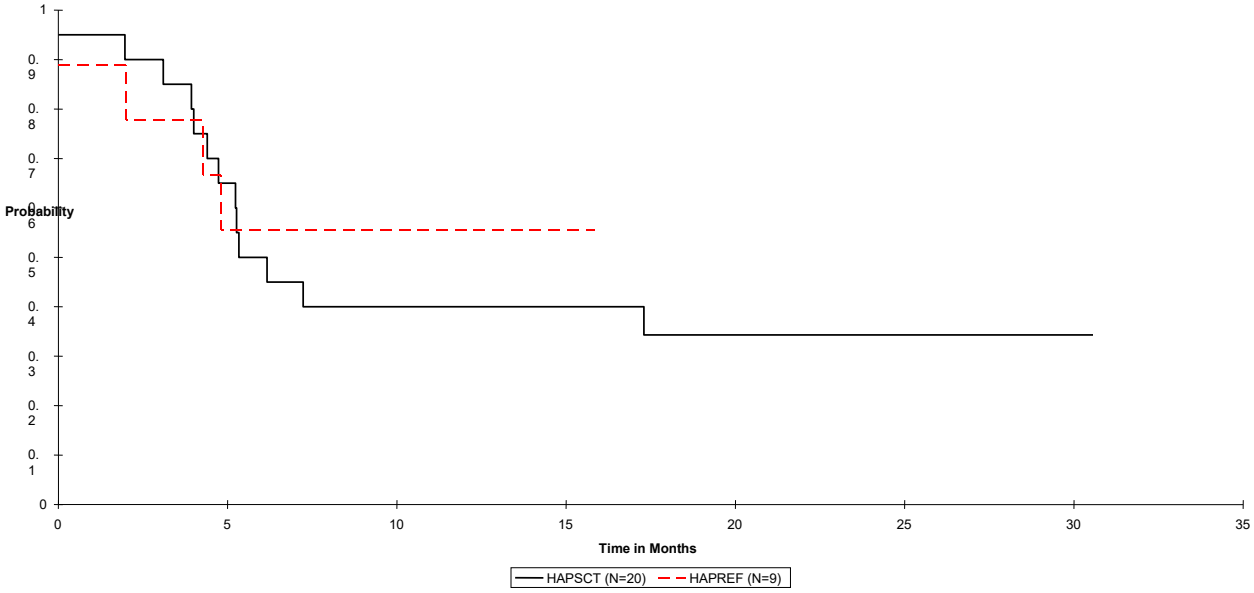

Table 6. Event-free survival in HAPSCT negative selection recipients and HAPREF Arm A participants (N = 29 total)

HAPSCT (N=20)						*HAPREF (N=9)*					
Time in Months	Risk	Fail	Cens	Prob	SE	Time in Months	Risk	Fail	Cens	Prob	SE
0	20	1	0	0.950	0.048	0	9	1	0	0.889	0.099
2	19	1	0	0.900	0.065	2	8	1	0	0.778	0.130
4	18	3	0	0.750	0.094	4	7	0	0	0.778	0.130
5	15	2	0	0.650	0.103	5	7	2	0	0.556	0.151
6	13	3	0	0.500	0.107	6	5	0	0	0.556	0.151
7	10	1	0	0.450	0.106	7	5	0	0	0.556	0.151

HAPSCT (N=20)						*HAPREF (N=9)*					
Time in Months	Risk	Fail	Cens	Prob	SE	Time in Months	Risk	Fail	Cens	Prob	SE
8	9	1	0	0.400	0.103	8	5	0	0	0.556	0.151
11	8	0	0	0.400	0.103	11	5	0	1	0.556	0.166
13	8	0	0	0.400	0.103	13	4	0	1	0.556	0.185
14	8	0	0	0.400	0.103	14	3	0	1	0.556	0.214
16	8	0	0	0.400	0.103	16	2	0	2	0.556	0.370
18	8	1	1	0.343	0.105						
19	6	0	1	0.343	0.113						
20	5	0	2	0.343	0.139						
25	3	0	1	0.343	0.160						
27	2	0	1	0.343	0.197						
31	1	0	1	0.343	0.278						

It may be noted that as of this time few research participants have follow-up of more than two years on HAPREF protocol and, hence, the estimate obtained may not be very reliable. Furthermore, since the HAPREF protocol was closed prematurely, it makes sense to assess the effectiveness of the proposed treatment plan by comparing the outcome (EFS) of this protocol to the outcome of HAPSCT. We would consider the findings to be of significant merit if we can demonstrate an improvement of at least a 20% in one-year EFS. The best approach would be to use the methods for analyzing survival data and propose the sample size based on comparing the EFS of the current protocol to the historical control group (HAPSCT). However, there is no good way to incorporate interim analysis to monitor for inferior EFS on the current protocol.

It may further be noted that on HAPSCT protocol there was no research participant censored within one-year of follow-up. Thus, with no censoring expected during one-year time period we can approximate the EFS at one-year using a Binomial distribution. That is, each research participant will be followed for one-year and will be counted as a success if the research participant survived without any events (death due to any cause or relapse). Denote by P the proportion of participants surviving at one-year without these events mentioned. Then using a more flexible phase II design such as the one proposed by Chen and Ng, we can assess if the proportion of participants surviving is in the desired range and if a larger clinical trial is warranted to validate the findings.[139]

Then setting up the problem in the binomial framework and assuming the proportion of research participants surviving without relapse at one-year on HAPSCT protocol to be roughly 40%, we would like to see if that proportion could be improved to 60%, i.e. an increase of 20%. Then using the optimal flexible phase II design proposed by Chen and Ng to detect an improvement of 20%, with 80% power and type I error control at level α=.05, we would enroll 12 to 19 participants in the first phase and 44 - 51 in the second.[139]

Because Amendment 3.0 includes a substantial change in conditioning (with the replacement of OKT3 by Campath-1H), we will restart counting of enrollment beginning from the first

patient enrolled since amendment 3.0. All the patients enrolled before Amendment 3.0 will be reported separately. The enrollment goal remains the same for this new cohort of patients as originally written, as does the statistical design.

The flexible stopping rules are provided in the following tables:

Table 7. Stopping rules for lack of efficacy (unacceptable lower rate of one-year event-free survival) based on the two-stage design

Reject the treatment plan if the number of research participants surviving at one-year					
P_0	P_1	$(\leq r_i/n_i)$	$(\leq R_j/N_j)$	AEN(P_0)	APET(P_0)
0.40	0.60	5/12-13, 6/14, 7/15-16, 8/17-19	22/44-45, 23/46-47, 24/48-49, 25/50, 26/51	25.01	0.71

Note: AEN(P_0) denotes the minimum average expected sample size under P_0; APET(P_0) denotes the probability of early termination at stage I under P_0

From the above Table 7 it is clear that the proposed stopping rules provide more flexibility since we can enroll research participants in a range of 12 - 19 and stop the trial if the number of participants surviving without any event is fewer than those listed in the table. For example, if at any point we see seven or more (≥ 7) relapses and/or deaths within one-year in the first 15- 16 then we would halt the enrollment and consider modifying the treatment plan and amending the protocol. The above stopping rule provides more flexibility in enrolling research participants and not requiring the study to be closed while the research participants are being followed for one-year. We expect to enroll roughly 12 - 15 participants per year with one-year of follow-up. The expected duration of the study will be about 5 years. However, there still exists a possibility that once 19 research participants have been enrolled we may have to temporarily close the trial to accrual while the research participants are being followed for one year. Only participants who initiate protocol therapy will be considered for the stopping rules. A participant who enrolls and subsequently dies prior to beginning protocol treatment would not count toward the efficacy stopping rule.

In addition, we will closely monitor for acute GVHD and TRM (Transplant Related Mortality as defined as death while in remission) for at least 100 days post-transplant to ensure and enhance safety of participants. Initially the stopping rule for toxicity excluded recipients who received more than 2 DLI in the first 100 days, as this was considered to be rare. However, after amendment 3.0 substituted Campath for OKT3 (due to OKT3 being no longer available), it became clear after the first 14 patients, that DLI were required more frequently. In addition, 4 of the first 14 patients evaluable at day 100 had developed Grade III-IV GVHD – and all of them had received multiple escalating DLI for post-transplant complications (see Section 4.9). Importantly, the use of DLI as described in this protocol has been largely successful (manuscript in preparation). So, continued use of DLI remains integral therapy on this protocol. Therefore with amendment 6.0, the toxicity stopping rules will include all patients who received protocol therapy (including DLI). Although the 4 patients above all developed grade III-IV GVHD within 100 days, the use of DLI can extend the time at which acute GVHD will occur. Therefore, any GVHD that occurs within the first 100 days post-transplant or within 100 days of their last cell therapy (including any stem cell boost and/or DLI) will count towards the GVHD stopping rule.

The initial stopping rule for toxicity included both TRM and GVHD together in the same monitoring rule. The stopping rule was based on exact upper 95% Blyth-Still-Casella confidence bounds that the combined rate of Grade III-IV GVHD and TRM was less than 40%. However with amendment 6.0, we have chosen to separate GVHD and TRM into separate stopping rules to allow more specific categorical assessment of each of these important toxicities. The stopping rules will each be based on the minimum exact lower 95% Blyth-Still-Casella confidence bounds that the rate of the specific toxicity (Grade III-IV GVHD and TRM respectively) do not exceed 20%. These current stopping rules are in place such that if there is statistical evidence that the rate of either toxicity is in excess of 20%, then the trial will be closed to further accrual.

Since we plan to treat 51 research participants, we will monitor the acute GVHD and TRM rate by setting up 4 interim evaluation time points as noted in Tables 8 and 9 below:

Table 8. Stopping rules for toxicities based on incidence of TRM

Number of participants enrolled	Number of TRM observed ≥	Minimum Exact Lower Confidence Bounds
19	7	0.209
30	11	0.221
40	13	0.204
51	16	0.208

Based on the above Table 8, if we observe 7 incidences of TRM within the first 100 days post-transplant in the first 19 evaluable research participants treated, then we will halt the trial and consider amending the protocol.

Table 9. Stopping rules for toxicities based on incidence of GVHD

Number of participants enrolled	Number of overall grade III-IV acute GVHD observed ≥	Minimum Exact Lower Confidence Bounds
19	7	0.209
30	11	0.221
40	13	0.204
51	16	0.208

Again, based on the above Table 9, if we observe 7 incidences of acute grade III-IV GVHD within the first 100 days post-transplant (or 100 days after the last cellular infusion) in the first 19 evaluable research participants treated, then we will halt the trial and consider amending the protocol. Again, it may be noted that as part of the treatment plan, some transplant recipients may require donor lymphocyte infusions (DLIs) or additional "boost" infusions (see Section 4.7 and 4.9 for additional details). If this happens within 100 days

post transplantation, then those research participants will have their evaluation period for acute grade III-IV GVHD extended up to 100 days post last cellular therapy (any stem cell boosts and/or DLI). It may be noted that the above stopping rules are "ad hoc" in nature. However, these are justified on the basis of stopping the trial if there is evidence to support a rate of acute grade III-IV GVHD or TRM higher than 20%. Also, the proposed stopping rules were deemed reasonable in PI's evaluation.

However, in estimating the incidence of overall grade III-IV acute GVHD or TRM, the estimates may be biased, as there are competing risks such as death due to causes other than acute GVHD or the transplant. Once the study is completed we will use more appropriate approaches, e.g. those based on Gray's approach to estimate the incidence of overall grade III-IV acute GVHD and provide confidence intervals.[140]

Secondary Objectives:

Objective 1.2.1 Once the data is collected the Kaplan-Meier estimates along with their confidence intervals will be provided for EFS, OS, and DFS. The terms EFS, DFS and OS are further defined below: LFU stands for "last follow-up date."

EFS = min(LFU, date of relapse, date of death due to any cause) - date of transplant and all participants surviving at the time of analysis would be considered as censored.

DFS = min(LFU, date of relapse, date of death due to relapse) - date of transplant and all participants surviving at the time of analysis and those who die due to other causes will be censored at the time of their event.

OS = min(LFU, date of death) – date of transplant and all patients surviving at the time of analysis will be considered as censored.

A historical comparison with the HAPSCT protocol will also be undertaken to see if the outcome on the current therapy has improved. Further a Cox's proportional hazards model will be used to assess the affect of several characteristics of the graft and disease status on the outcome measures such as EFS, DFS and OS.[141]

Objective 1.2.2 and 1.2.3. The estimates of cumulative incidence of relapse, acute and chronic GVHD will be estimated using Prentice's approach and compared with the historical HAPSCT data using the approaches proposed in Gray and Fine and Gray.[140, 142,143,157]

Objective 1.2.4. The estimates of non-hematologic regimen related toxicity and regimen related mortality in first 100 days for the 2 groups will be estimated and compared using methods for comparing 2 bionomial distributions discussed in Agresti.[144]

Objective 1.3.1. The biologic significance of soluble interleukin-2 receptor and immunologic state to predict the development of acute and chronic GVHD will be evaluated using the method proposed in Fine and Gray and implemented using an in house SAS Macro.[143]

11.0 DATA ACQUISITION AND QUALITY ASSURANCE MONITORING

11.1 Enrollment on study

After verification of subject eligibility (donor and transplant recipient), potential participants will be registered by completing the eligibility checklist and faxing the

completed Eligibility Checklists to the Central Protocol and Data Monitoring Office (CPDMO) at 595-6265. Follow with a phone call to (901) 595-2568 to ensure that the fax has been received. Eligibility will be reviewed and entered into the institutional database, and a research participant-specific consent form will be generated. The consents, protocol, and protocol standard order set will be delivered to the area designated on the Eligibility Checklist(s). The signed consent forms must be faxed to the CPDMO for entry into the centralized database.

Central Protocol Office Hours. The CPDMO is staffed 7:30 am to 6:00 pm CST, Monday through Friday (excluding holidays). A staff member is available by pager 8:00 am to 5:00 pm on weekends and weekday holidays. To reach the person on call during these hours, call 901-595-3578, wait for the prompt, and enter pager #2173, followed by the telephone number to which the call should be returned.

11.2 Data submission

The St. Jude Cancer Center Clinical Research Associates and Research Nurses with the Division of Bone Marrow Transplant and Cellular Therapy will be responsible for protocol compliance assurance/monitoring as well as clinical and safety data collection. The PI will be responsible for review of case report forms for accuracy and completeness prior to entry into the secure divisional database. Data such as laboratory values will also be transferred directly into the database from the institutional Millennium data system.

11.3 Quality assurance monitoring

This protocol will be monitored for safety and data as per the St. Jude institutional Data Safety Monitoring Plan, dated August 17, 2001. Source document verification of eligibility for all patients will be performed within 2 weeks of completion of enrollment. This will include verification of appropriate documentation of consent. Monitoring of timeliness of adverse and serious AE reporting will be done as events are reported. Monitoring of modified Clinical Data Update System (CDUS) elements, AE reporting, data required for primary objectives, and compliance with the conduct of the protocol will be performed on a semiannual (every 6 months) or an as needed basis.

12.0 OBTAINING INFORMED CONSENT

The ongoing informed consent process will be carried out per the policies and procedures put forth in the St. Jude Investigator's Handbook for Clinical Research (http://home.web.stjude.org/ clinical_research/administration/doc/handbook.pdf). The PI or physician sub-investigator will conduct the signature authorization portion of the consent process. Authorization for the recipient procedure will be conducted in the presence of an independent witness such as the St. Jude Ombudsperson/Research Participant Advocate or designee, a professional staff member from the Department of Nursing, or Social Work.

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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 Graft-vs.-Host Disease[164]

Organ Staging:

Organ		Stage 0	Stage 1	Stage 2	Stage 3	Stage 4
Skin		No rash	Rash on < 25% body surface area[a]	Rash ≥25% to ≤ 50%	Rash >50% generalized erythroderma	Plus bullae and desquamation
Gastrointestinal	Lower GI	Diarrhea < 500 mL/day	Diarrhea 501 to 1000 mL/day[c]	Diarrhea 1001 to 1500 mL/day	Diarrhea >1500 mL/day	Severe abdominal pain with or without ileus
	Upper GI		Persistent nausea[d]			
Liver		Bilirubin < 2.0 mg/dl	Bilirubin 2.1 to 3.0 mg/dl[b]	Bilirubin 3.1 to 6.0 mg/dl	Bilirubin 6.1 to 15 mg/dl	Bilirubin 15 mg/dl

Overall Grading for acute GVHD

Grade	Skin	Liver	Gut
I	Stage 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III		Stage 2-3 or	Stage2-4
IV[f]	Stage 4 or	Stage 4	

a) Use "Rule of Nines" or burn chart to determine extent of rash.

b) Range given as total bilirubin. Downgrade one stage if additional cause of elevated bilirubin is documented.

c) Volume of diarrhea applies to adults. For pediatric patients, the volume of diarrhea should be based on body surface area. Downgrade one stage if additional cause of diarrhea has been documented.

d) Persistent nausea with histological evidence of GVHD in stomach or duodenum.

e) Criteria for grading given as minimum degree of organ involvement required to confer that grade.

f) Grade IV may also include lesser organ involvement but with extreme decrease in performance status.

APPENDIX B (continued)

Notes:

- Liver GVHD is downgraded one stage if an additional cause(s) of an elevated bilirubin is documented.
- Gut GVHD is downgraded one stage if additional cause(s) of increased diarrhea/loose stool volume is documented.
- Specified stool volumes noted for gut GVHD applies to adult range body weights. For pediatric patients, the volume of stool should generally be based on body surface area.
- Stool/urine mixture cannot be considered total stool volume. Stool volume needs to be unmixed stool without urine volume factored into volume total.
- Abdominal pains secondary to other etiologies need to be ruled out or factored into abdominal GVHD assessments accordingly.
- Overall clinical performance may also be considered in the clinical grading of severity of acute GVHD.

CRITERIA FOR GRADING CHRONIC GVHD

Chronic GVHD typically occurs after the day +100 time point or 100 days post stem cell boost as applicable. The diagnosis is determined by the transplant physician based on clinical assessment and may be supported by pathology findings.

Staging of Chronic GVHD:

Limited - Localized skin and/or hepatic dysfunction.

Extensive: One or more of the following (as clinically judged by a physician and deemed as chronic GVHD by the PI):

- Generalized skin involvement
- Liver histology showing chronic aggressive hepatitis, bridging necrosis and/or cirrhosis.
- Eye dryness with Schirmer's test <5 mm wetting
- Oral: involvement of salivary glands or oral mucosa.
- Other: another target organ involvement.

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 10 Documenting and Reporting of Adverse Events) will provide guidance on the evaluation, collection and reporting of adverse events for this clinical trial. The current version of this document, as well as ongoing updates, can be located at the following website: http://home.stjude.org/bmt/Pages/policies-research.aspx

APPENDIX D

Recommended testing and evaluation schedule

STANDARD OF CARE STUDIES	SAMPLE	VOLUME	PRE	MONTH 1	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
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
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.
- 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 RECIPIENT

RESEARCH STUDIES	SAMPLE	VOLUME	PRE	MONTH 1	MONTH 2	MONTH 3	MONTH 6	MONTH 12
VBETA/TREC RESEARCH	PB	17 mL	X			X	X	X
LYMPHOCYTE PHENOTYPES RESEARCH	PB	17 mL	X	X	X	X	X	X
SOLUBLE INTERLEUKIN-2	PB	2 mL	X	Weekly starting DAY -7, until approximately DAY 100				
TUMOR NECROSIS FACTOR	PB	3 mL	X	Weekly starting DAY -7, until approximately DAY 100				

- VBETA/TREC Research and Lymphocyte Phenotypes Research results will be maintained in the Leung laboratory database.
- For RESEARCH studies, the posted volumes are the minimum volumes required to perform the respective protocol evaluations.

Optional Campath-1H pharmacokinetic testing schedule for RECIPIENT

RESEARCH STUDIES	SAMPLE	VOLUME	DAY -10	DAY -8	DAY -3	WEEK 1	WEEK 2	WEEK 4	WEEK 8	WEEK 16
ALEMTUZUMAB HIFLEX (CAMPTH-1H)	PB	2 mL	X	X	X	X	X	X	X	X

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
T cell and NK cell number and function	Lymphocyte Phenotypes Research = 17 mL

- All donor research testing to be collected prior to stem cell collection – preferably prior to growth factor administration. These optional research tests may be collected at separate times.

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 located at the following website: http://home.web.stjude.org /bone_marrow/clinicalHome.shtml.

APPENDIX F

Dose modification and off treatment criteria for Campath-1H toxicity

Recommended pre-medication to be given with Campath-1H:

First doses should be timed approximately 30 minutes prior to daily Campath-1H dose.
Diphenhydramine: 1mg/kg IV or PO q 8hr (max dose 50mg)
Acetaminophen: 10-15mg/kg PO q 6hr (max 4gm/day)
Hydrocortisone: 2mg/kg IV q 6 hr (when Campath is given SQ, may use 1mg/kg q 6 hr)
Meperidine: 0.5mg/kg IV q 4hr PRN rigors

Modifications in the event of an adverse reaction during test dose administration:

- If the reaction is mild (Grade 1), such that no intervention is required, and the infusion is not interrupted; proceed with subsequent dosing as planned. Examples of grade 1 reactions are: Transient flushing, rash, and/or low grade fever that resolve promptly and without intervention.
- If the reaction requires therapy (such as additional antihistamine or acetaminophen) or a brief interruption of infusion, and responds promptly (Grade 2); proceed with dosing as planned. Depending on the seriousness of the adverse reaction, consider alteration of premedication (e.g., change steroid to methylprednisolone 2mg/kg q 6hr), and/or a reduction in the planned infusion rate.
- If the reaction is prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion) but improves prior to next dose (24hr), recurs following initial improvement but improves prior to next dose, or is serious (i.e. symptomatic bronchospasm, significant hypotension) but improves with intervention (Grade 3); notify the PI and make the following modifications: At a minimum, increase dose and/or frequency of supportive medications (corticosteroids, antihistamines, acetaminophen), and ensure infusion is begun at a reduced rate. Depending on the seriousness of the reaction, consider discontinuation of Campath-1H with substitution of ATG as alternative therapy.
- If the reaction is life threatening (Grade 4), notify the PI, discontinue Campath-1H, and use ATG as alternative therapy if possible.

For adverse reactions which occur during/following standard dose administration:

- If the reaction is mild (Grade 1), such that no intervention is required, and the infusion is not interrupted; proceed with subsequent dosing as planned. Examples of grade 1 reactions are: Transient flushing, rash, or a low grade fever that resolve quickly without intervention.
- If the reaction requires therapy (such as additional antihistamines or acetaminophen) or a brief interruption of infusion, and responds promptly (Grade 2); may proceed with dosing as planned. Consider alteration of premedication (e.g, change to methylprednisolone), and/or a reduction in the planned infusion rate.

- If the reaction is prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion) but improves prior to next dose (24hr), recurs following initial improvement but improves prior to next dose, or is serious (i.e. symptomatic bronchospasm, significant hypotension) but improves with intervention (Grade 3); notify the PI and make the following modifications: Increase dose and/or reduce interval on supportive medications (corticosteroids, antihistamines, acetaminophen). Ensure infusion is begun at a reduced rate. No further dose escalation is permitted, and depending on the seriousness of the reaction consider a reduction in subsequent doses.
- If the reaction is prolonged and does NOT improve with intervention prior to the next scheduled dose (Grade 3), notify the PI, discontinue Campath-1H, and use ATG as alternative therapy if possible.
- If the reaction is life threatening or requires intensive support (Grade 4), notify the PI, discontinue Campath-1H, and use ATG as alternative therapy if possible. May consider reduction of subsequent dosing and escalation of supporting medications, ONLY if a lower dose was previously tolerated, AND symptoms resolve entirely prior to next dose, AND no suitable alternative is available.

Substitution of ATG if Campath-1H intolerance:

If the patient received less than 50% of the planned Campath-1H dose, efforts should be made to give ATG in substitute.
Typically rabbit ATG should be given as 3mg/kg/day for 3 days.
In the event the patient received less than 50% of the planned Campath dose, and ATG is contraindicated, the patient will be removed from study.

APPENDIX G

Responsibility for Campth-1H PK collection and analysis at St. Jude

Delegation of responsibility of PK sample collection will be as follows:

1. Blood collection: St. Jude nurse
2. Plasma extraction: St. Jude PK technician
3. Freeze and store plasma: St. Jude PK technician
4. Sample log: St. Jude PK technician
5. Shipment to Genzyme: St. Jude PK technician (2 – 5 under the supervision of the BMT and CT Pharmacy sub-investigators - Dr. Cross).
6. Bioanalysis: Genzyme
7. Interpretation of raw data: Genzyme will provide a written report of results to be emailed to Dr. Brandon Triplett in pdf format.

PK TESTING MATERIALS AND INSTRUCTIONS FOR **U.S. & CANADIAN SITES** OR COLLECTION, PROCESSING, PACKAGING & SHIPMENT OF BLOOD SAMPLES

SAMPLE COLLECTION MATERIALS

Supplied by Genzyme

- Instructions for handling/processing
- 2 x 5 mL serum separator tubes with gold hemoguard
- 4 x 2 mL plastic freezer storage tubes (cryovials)
- 6 labels for the serum separator tube(s) and cryovials
- 2 plastic transfer pipettes
- 4 sheets of absorbent material
- Rubber band
- 1 sample-shipping container pre-labeled with “BIOHAZARD”
- 2 cold packs
- Styrofoam Sample Collection Box suitable for shipping
- Exterior Cardboard Shipping Box
- Pre-addressed FedEx airway bills (one U.S. Airbill and one International Air Waybill)
- Antibody Testing Requisition Form
- Plastic bag for placement of Antibody Testing Requisition Form
- FedEx Diagnostic Envelope with IATA Compliance Label for placement of the box for transit
- Pro-Forma Invoice (for Canadian Sites Only)

Supplied by Site

- Freezer capable of freezing to preferably ≤ -60° C (≤ -20° C is acceptable)
  - If unavailable, see alternative option (pg 4).
- Centrifuge (capable of 2000-3100 rpm)
  - If unavailable, see alternative option for collection and shipment of whole blood (pg 5).
- Blood collection materials (gloves, alcohol swabs, tourniquets, needles)
- Packing tape

Please adhere to the following instructions as samples that are mishandled may result in sample degradation. Samples that are incorrectly labelled will not be tested.

Blood Collection and Processing for FROZEN SPUN SERUM SAMPLES
Fill the 2 x 5mL (for adults) or 1 x 5mL (for pediatric patients) serum separator blood collection tube(s). Samples should be drawn prior to the Campath infusion.
Label the serum separator blood collection tube(s) using the labels provided. Label with Genzyme Product name (i.e.- Campath), patient initials, patient DOB (dd/mmm/yyyy – e.g., 10/Mar/1968), sample collection date (dd/mmm/yyyy), sample collection time (24 hour clock: 2:00 PM = 14:00 hours).
Allow blood to clot between 30 – 60 minutes (not longer) at room temperature.
To separate serum, centrifuge blood sample at 2˚C - 8˚C (if available) at 2000-3100 rpm for 10-15 minutes.
Transfer the cell-free serum, in approximate equal aliquots, into the 4 x 2mL freezer vials (cryovials) provided (2 cryovials for pediatric patients).
Label the cryovials with the labels provided. Label with Product name (i.e.- Campath), patient initials, patient DOB (dd/mmm/yyyy - e.g. 10/Mar/1968)), patient Registry ID#, sample type (e.g., serum), sample collection date (dd/mmm/yyyy), sample collection time (24 hour clock: 2:00 PM = 14:00 hours) and Physician name.
Freeze all cryovials in the upright position at ≤ -60˚C (≤ -20˚C is acceptable) for a minimum of 2 hours. Keep all vials frozen until ready to ship.

Blood Packaging and Shipping for FROZEN *SPUN SERUM SAMPLES*
*Contact information: For questions contact **1-800-745-4447** or **1-617-768-9000** (option 2)*
Samples may be shipped after a minimum of 2 hours of freezing. Multiple patient samples may be shipped together in the same box with proper sample labeling only. Cryovials must remain frozen at all times. DO NOT ALLOW THE SAMPLES TO THAW during the shipping preparations described below.
*Samples should be shipped Monday-Thursday (Next Day Delivery) from U.S. and Canadian sites, as long as **WEEKDAY DELIVERY TO GENZYME** is assured (Canadian sites should ship Monday-Wednesday, Thursday only in an emergency).*
Complete the Campath Testing Requisition Form completely. Retain the white copy and insert the yellow and pink copies into the plastic paperwork bag provided.
When ready for shipping, remove the cryovials from the freezer and wrap each cryovial with one piece of absorbent material and bundle all cryovials with a rubber band.
Place the wrapped cryovials in the sample-shipping container labeled "Biohazard."
Place the frozen cold packs and sample-shipping container labeled "Biohazard" (containing the cryovials) into the Styrofoam shipping box. [NOTE: Cold packs must be frozen for a minimum of 48 hours prior to shipping.]
Place the plastic bag containing the Campath Testing Requisition Form on top of the frozen cold packs.
Place the cover on the box making sure it is flush with the top of the wall liner, allowing the box to close completely. If not done properly, the adequate temperature to maintain the frozen samples may not occur. Close the box with packing tape.
Place the Styrofoam shipping box into the exterior cardboard box and place the cardboard box into the supplied FedEx shipping envelope.

Blood Packaging and Shipping for FROZEN SPUN SERUM, Continued	
Complete all the necessary information requested on the FedEx airway bill, specifically: • Complete Section 1: Enter the sender's information (you do not need to provide a FedEx account #) • Complete Section 6: "Special Handling" by checking "No." **NOTE: These must be completed or the package will not be delivered.** • If sending from U.S., use the enclosed domestic US airbill • If sending from CANADA, use the enclosed International airbill	
Attach the pre-addressed airway bill to the envelope provided.	
***Canadian sites only**: complete and include 4 copies of the Pro-Forma invoice with the FedEx airway bill.*	
*Call FedEx at **1-800-463-3339** to arrange sample pick-up.*	
*Notify Genzyme Clinical Specialty Labs of the pending shipment with the tracking number ;via; TEL: 800-745-4447, option 0, ext 2*2449, e-mail **CSLtesting@genzyme.com** or FAX: 508-270-2023 .	
All U.S. and Canadian sites ship samples via FedEx to:	***Genzyme Corporation - Clinical Specialty Laboratory*** ***One Mountain Road*** ***Framingham, MA 01701-9322 USA*** ***Telephone: 1-508-270-2449***