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BLOOD AND MARROW
TRANSPLANT
CLINICAL TRIALS NETWORK

A Randomized, Multi-Center, Phase III Trial of Calcineurin Inhibitor-Free Interventions for Prevention of Graft-versus-Host Disease

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PROTOCOL SYNOPSIS

A Randomized, Multi-Center, Phase III Trial of Calcineurin Inhibitor-Free Interventions for Prevention of Graft-versus Host-Disease

Co-Principal Investigators: Leo Luznik, Miguel-Angel Perales, Marcelo Pasquini

Study Design: The study is designed as a three arm randomized Phase III, multicenter trial comparing two calcineurin inhibitor (CNI)-free strategies for GVHD prophylaxis to standard calcineurin inhibitor tacrolimus and methotrexate (Tac/Mtx) in patients with acute leukemia or myelodysplasia undergoing myeloablative conditioning hematopoietic stem cell transplantation.

Primary Objective: The primary objective of the randomized trial is to compare chronic GVHD/relapse-free survival [CRFS] as a time to event endpoint after hematopoietic stem cell transplant (HSCT) between each of the CNI-free interventions and a Tac/Mtx control.

Secondary Objectives: Secondary objectives are: comparison of rates of grade II-IV and III-IV acute GVHD, chronic GVHD, chronic GVHD-free survival, immunosuppression-free survival at one year, primary and secondary graft failure, neutrophil and platelet engraftment, disease relapse, transplant-related mortality, rates of Grade ≥ 3 toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0; incidence of CMV and EBV reactivation, incidence of infections; immune reconstitution, quality of life and overall survival.

Eligibility Criteria: Patients ≥ 1 year and < 66 years undergoing HSCT for treatment of acute leukemia in morphologic complete remission with or without hematologic recovery or myelodysplasia with < 5% blasts in the marrow and no circulating blasts, and who are eligible for a myeloablative allogeneic transplant. Patients with CMML must have a WBC count ≤ 10,000 cells/µL and < 5% blasts in the marrow. Patients with ≥ 5% blasts due to a regenerating marrow must contact the protocol chairs for review. Patients must have a related or unrelated donor. Related donor must be an 8/8 match for HLA-A, -B and -C at intermediate (or higher) resolution, and -DRB1 at high resolution using DNA-based typing. Pediatric related donors must weigh ≥ 25.0 kg, must have adequate peripheral venous catheter access for leukapheresis or must agree to placement of a central catheter, must be willing to (1) donate bone marrow and (2) receive G-CSF

followed by donation of peripheral blood stem cells (product to be determined by randomization post enrollment), and must meet institutional criteria for donation. Unrelated donor must be an 8/8 match at HLA-A, -B, -C and –DRB1 at high resolution using DNA-based typing. Unrelated donor must be medically eligible to donate according to NMDP (or equivalent donor search organization) criteria. At time of enrollment, the donor should not have any known preferences or contraindications to donate bone marrow or peripheral blood stem cells.

Treatment Description: Patients will be randomized to receive one of the three specified interventions: 1) CD34 selected T-cell depleted peripheral blood stem cell (PBSC) graft; 2) unmanipulated bone marrow (BM) graft followed by cyclophosphamide (Cy) 50mg/kg Day +3 and +4 post HSCT; or, 3) unmanipulated BM graft with Tac/Mtx GVHD prophylaxis. Tac will be maintained at therapeutic doses for a minimum of 90 days. Methotrexate will be dosed at 10-15mg/m^2 for a maximum of 4 doses post-transplant.

Accrual Objective: The clinical trial will enroll 345 patients or 115 per arm, in an adaptive design for futility evaluated at time of interim analysis.

Accrual Period: The estimated accrual period is 42 months.

Study Duration: Patients will be followed for 2 years following hematopoietic cell transplantation.

Interim Analysis: No formal interim analyses for efficacy will be used. There is also not included in the design an option for closure of the control group while keeping the two treatment arms open, in the event that at least one of the treatments demonstrate early efficacy. Interim analyses for futility will be conducted at times coincident with regularly scheduled meetings of the DSMB, starting when approximately 45-50% of the targeted number of events have been observed.

Stopping Guidelines: Monitoring of a key safety endpoint (mortality) will be conducted monthly up to 100 days post-randomization separately in each of the three treatment arms. At least three events must be observed in order to trigger review.

Correlative Studies: Comparison of immune reconstitution using a panel of clinically available tests across all treatment arms. Advanced immune reconstitution assays.

STUDY SCHEMA

Aim: To determine if either CNI-free approach improves the rate of chronic GVHD and relapse free survival after transplant compared to standard Tac/Mtx control.

Inclusion Criteria

1. Males and females aged ≥ 1.0 year and < 66.0 years
2. Patients with acute leukemia, in morphologic complete remission with or without hematologic recovery or myelodysplasia with < 5% blasts in the marrow and no circulating blasts. Patients with CMML must have a WBC count ≤10,000 cells/μL and < 5% blasts in the marrow. Patients with ≥ 5% blasts due to a regenerating marrow must contact the protocol chairs for review.
3. Planned myeloablative conditioning regimen (see eligible regimens in Table 2.4).
4. Patients must have a related or unrelated donor as follows:
   a. Related donor must be an 8/8 match for HLA-A, -B and -C at intermediate (or higher) resolution, and -DRB1 at high resolution using DNA-based typing. Pediatric related donors must weigh ≥ 25.0 kg, must have adequate peripheral venous catheter access for leukapheresis or must agree to placement of a central catheter, must be willing to (1) donate bone marrow and (2) receive G-CSF followed by donation of peripheral blood stem cells (product to be determined by randomization post enrollment), and must meet institutional criteria for donation.
   b. Unrelated donor must be an 8/8 match at HLA-A, -B, -C and –DRB1 at high resolution using DNA-based typing. Unrelated donor must be medically eligible to donate according to NMDP (or equivalent donor search organization) criteria. At time of enrollment, the donor should not have any known preferences or contraindications to donate bone marrow or peripheral blood stem cells.
      i. Selection of unrelated donors is to be performed according to institutional practice. It is recommended that the time from collection to initiation of the cell processing be considered when prioritizing donors as data shows better results for CD34 selection when processing is started within 36 hours of the end of collection as indicated in section 2.5.1.2.
5. Cardiac function: Ejection fraction at rest ≥ 45% or shortening fraction of ≥ 27% by echocardiogram or radionuclide scan (MUGA**)**.
6. Estimated creatinine clearance (for patients > 12 years) greater than 50 mL/minute (using the Cockcroft-Gault

Exclusion Criteria

1. Prior autologous or allogeneic hematopoietic stem cell transplant
2. Karnofsky or Lansky Performance Score < 70%
3. Active CNS involvement by malignant cells
4. Patients with uncontrolled bacterial, viral or fungal infections (currently taking medication and with progression or no clinical improvement) at time of enrollment
5. Presence of fluid collection (ascites, pleural or pericardial effusion) that interferes with methotrexate clearance or makes methotrexate use contraindicated
6. Patients seropositive for HIV-1 or -2
7. Patients seropositive for HTLV-I or –II
8. Patients with active Hepatitis B or C viral replication by PCR
9. Documented allergy to iron dextran or murine proteins
10. Women who are pregnant (positive serum or urine βHCG) or breastfeeding
11. Females of childbearing potential (FCBP) or men who have sexual contact with FCBP unwilling to use 2 effective forms of birth control or abstinence for one year after transplantation
12. History of uncontrolled autoimmune disease or on active treatment
13. Patient with prior malignancies, except resected non-melanoma or treated cervical carcinoma in situ. Cancer treated with curative intent ≥ 5 years previously will be allowed. Cancer treated with curative intent < 5 years previously will not be allowed unless approved by the Protocol Officer or one of the Protocol Chairs.
14. Patient unable to comply with the treatment protocol, including appropriate supportive care, follow-up and research tests
15. Planned post-transplant maintenance therapy except for FLT3 inhibitors or TKIs
    a. Must be declared prior to randomization

formula and actual body weight); for pediatric patients (≥ 1 year to 12 years), GFR estimated by the updated Schwartz formula ≥ 90 ml/min/1.73 m^2. If the estimated creatinine clearance is < 90 ml/min/1.73 m^2, then renal function must be measured by 24-hour creatinine clearance or nuclear GFR, and must be > 70 mL/minute/1.73m^2. 7. Pulmonary function: DLCO ≥50% (adjusted for hemoglobin), and FVC and FEV1≥50%; for children who are unable to perform for PFTs due to age or developmental ability, there must be no evidence of dyspnea and no need for supplemental oxygen as evidenced by O_2 saturation ≥ 92.0% on room air. 8. Liver function: total bilirubin < 2x the upper limit of normal (unless elevated bilirubin is attributed to Gilbert's Syndrome) and ALT/AST < 2.5x the upper normal limit. 9. Signed informed consent	16. Planned use of cryopreserved hematopoietic stem cells 17. *German centers only:* Treatment with any known non-marketed drug substance or experimental therapy within 5 terminal half lives or 4 weeks prior to enrollment, whichever is longer, or participation in any other interventional clinical study.

Primary endpoint:
- Chronic GVHD/relapse-free survival (CRFS): this time to event outcome is defined as moderate to severe chronic GVHD by the NIH consensus criteria, disease relapse, or death by any cause.

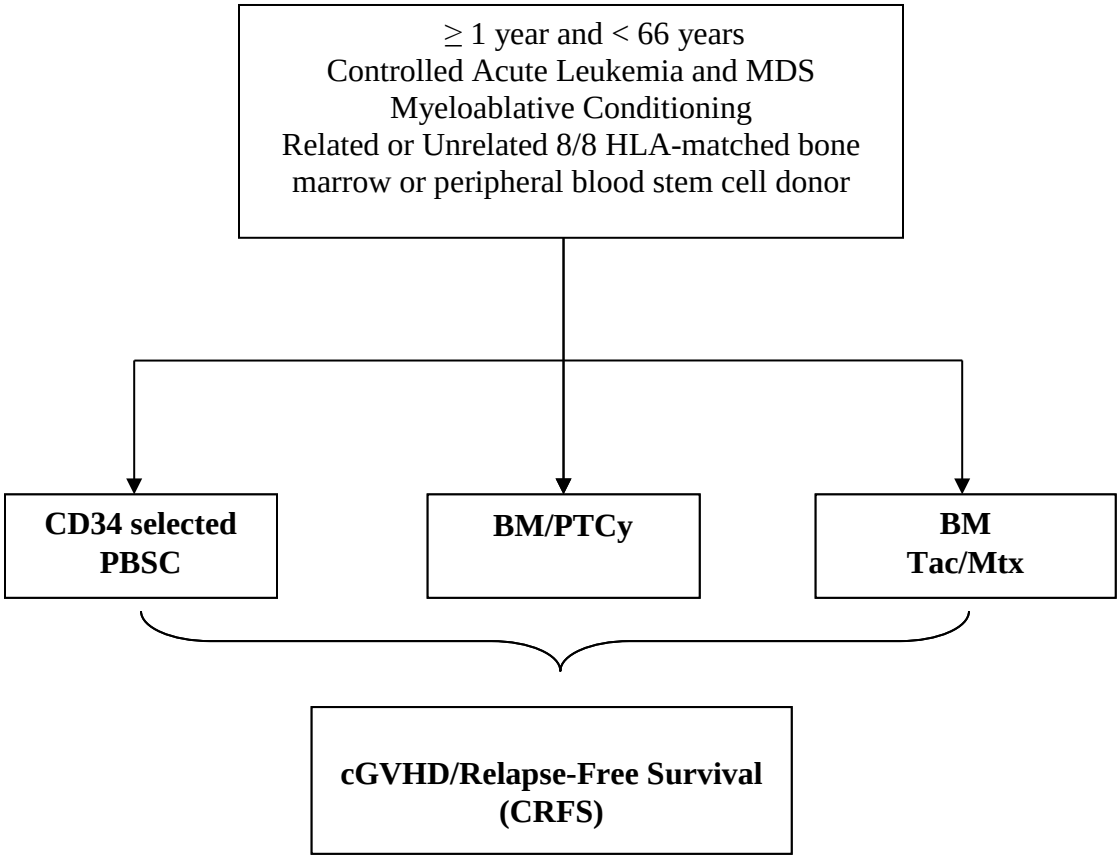
Secondary endpoints:
- Grades II-IV and III-IV acute GVHD
- Chronic GVHD
- Chronic GVHD-free survival
- Immunosuppression-free survival at 1 year
- Hematologic recovery
- Primary and secondary graft failure
- Immune reconstitution
- Disease relapse
- Transplant-related mortality
- Toxicity (SOS and IPS) and rates of infections (CMV and EBV reactivation)
- Relapse-Free and overall survival
- Quality of life

Allowed Myeloablative Conditioning Regimens		
CD 34 Selected Arm	PTCy Arm	Control Arm
Cy/TBI/Thiotepa/ ATG Bu/Mel/Flu/ATG	Cy/TBI Flu/Bu Bu/Cy	Cy/TBI TBI/Etoposide Flu/Bu Bu/Cy

***Busulfan dose >8mg/kg or IV equivalent**

***Immunosuppression Taper*:**
Patients without GVHD
Tacrolimus
- Taper to initiate at least by Day 90 and completely off at Day 180.

Outline of Treatment Plan



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CHAPTER 1

1. BACKGROUND AND RATIONALE

1.1. Introduction

Chronic graft versus host disease (GVHD) is a devastating complication that affects hematopoietic cell transplant (HCT) survivors and may offset the associated benefits of reducing rates of post-transplant disease relapse.[1, 2] GVHD prevention regimens are a required component of any allogeneic HCT approach, and most commonly use a combination of immunosuppressive agents given for the first 6 months after transplant. Often, patients develop GVHD and continue on these agents for much longer periods. The combination of calcineurin inhibitors (tacrolimus and cyclosporine A) with methotrexate (MTX) is the most common GVHD prophylaxis used worldwide in the context of myeloablative conditioning transplants. This regimen, implemented more than 3 decades ago, demonstrated better control of acute GVHD.[3,4] However, this strategy is ineffective against chronic GVHD. Management of chronic GVHD remains a challenge and it has become a significant health problem in transplant survivors with more frequent use of mobilized peripheral blood stem cells. Additionally, several issues arise with this treatment approach including: 1) MTX accentuated risk of conditioning regimen-related oral mucositis and renal toxicity; 2) Increased risk of thrombotic microangiopathy due to CNI; 3) Requirement for chronic monitoring of CNI serum levels; and, 4) Chronic immunosuppression is not a conducive platform for post HCT cellular therapies for treatment or prevention of infections or disease relapse.

In vivo graft manipulation with anti thymocyte globulin (ATG)[5, 6] or alemtuzumab,[7, 8, 9] ex vivo graft manipulation with CD34 selection and T-cell depletion[10] and, most recently, use of high doses of cyclophosphamide after transplant (PTCy) are strategies that are associated with lower rates of chronic GVHD. In addition a benchmark analysis of novel approaches to prevent GVHD using data from the Center for International Blood and Marrow Transplantation Research (CIBMTR) identified two strategies for T-cell depletion using either CD34-selection or administration of post-transplantation Cy cyclophosphamide (PTCy) as the most effective in preventing both acute and chronic GVHD after HCT. This clinical trial further explores these approaches to prevent chronic GVHD that have the additional benefit of not requiring prolonged immunosuppression.

1.2. CD34+ Selection and T-Cell Depletion

The recognition that GVHD is mediated by donor derived T-cells led to pre-clinical and clinical exploration of T-cell depletion to reduce the risk of GVHD. The use of *ex vivo* T-cell depleted (TCD) grafts has significantly reduced the risk of GVHD without the need for post-transplant immunosuppression.[11, 12, 13, 14, 15, 16, 17, 18] Although several different approaches have been used over the years, more recently, removal of T-cells from the graft has been achieved through positive selection of CD34+ cells using immunomagnetic beads.[12, 13, 14, 18, 19, 20, 21] In earlier studies, CD34+ selection of PBSCs was performed on the ISOLEX 300i magnetic cell selection system (Baxter,

Deerfield, IL), followed by E-rosetting.[12, 13, 20] Current studies are using super-paramagnetic particle-conjugated antibodies on the CliniMACS® Cell Separation System (Miltenyi Biotec, Gladbach, Germany) for CD34+ selection.[14, 20, 21] The CliniMACS® System can also be used to negatively select grafts through depletion of CD3 and CD19 cells or depletion of TCRαβ T-cells.[22] When assessing data from clinical trials reporting the use of TCD grafts it is critical to review the specific approach used. The degree of T-cell depletion is a particularly critical factor. In recipients of TCD marrow grafts from HLA-identical donors, the risk of GVHD was shown to increase if the graft contained > $1x10^5$ T-cells/kg.[23] For example, the CliniMACS® System can achieve a 5-log reduction in T-cells, whereas the ISOLEX 300i system (no longer available in the US) achieves a 3.5- to 5 log reduction, depending on the settings, and may require additional T-cell depletion through E-rosetting in the case of the lower log reduction. The CliniMACS® CD34 Reagent System was approved as a humanitarian device and authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this use has not been demonstrated.

1.2.1. Both Acute and Chronic GVHD are Decreased

The main goal of using a TCD or CD34-selected grafts is to reduce the risk of both acute and chronic GVHD. This result has been achieved in most studies, although to varying degrees. The risk of GVHD generally has correlated with the extent of T-cell depletion. The risk of both acute and chronic GVHD decreases significantly when the degree of T-cell depletion is 3-log using bone marrow or 4-5 log using PBSC.[11, 12, 13, 14, 18, 24, 25] For example, studies performed at MSKCC that achieved a 5-log reduction in T-cells using CD34-selection followed by E-rosetting have reported incidences of acute GVHD (limited to grade II) of 8%, and chronic GVHD of 9% in recipients of matched related grafts and incidences of acute and chronic GVHD of 9% and 29%, respectively, in recipients of matched unrelated donors.[12, 13] None of the patients received GVHD prophylaxis beyond T-cell depletion of the graft. Results from single-center studies have been validated in BMT CTN 0303 in which 44 patients with AML in CR1 or CR2 were conditioned with TBI, thiotepa, and cyclophosphamide with rabbit ATG followed by a single step TCD, PBSC allogeneic graft from an HLA-identical sibling donor. The incidence of acute GVHD grade II-IV was 22.7%, and the incidence of extensive chronic GVHD was 6.8% at 24 months.[14] Importantly, these results are in the setting of peripheral blood stem cell grafts.

1.2.2. CD34 Selection Impact on Graft Rejection and Relapse

A potential consequence of T-cell depletion is a higher risk of graft rejection. In fact, early studies of T-cell depletion were associated with higher rates of graft rejection than observed in recipients of conventional grafts, with reported graft failure rates as high as 27%.[26, 27, 28] These clinical results confirmed pre-clinical data that donor-derived T-cells facilitate engraftment. Following modifications of the conditioning regimen, and in particular the use of ATG to promote engraftment, several centers have reported consistent engraftment with TCD grafts using a variety of approaches for T-cell depletion, including CD34-selection.[11, 12, 13, 14, 24, 25]

Another potential and significant limitation of T-cell depleting the graft is an increase in relapse, due to elimination of the recognition of the tumor by donor-derived T-cells, the so-called graft

versus leukemia (GVL) effect. This was illustrated in patients with CML, where a retrospective study of 46 patients who underwent TCD transplants were compared to 40 patients who had conventional grafts.[29] The 3-year probability of relapse was higher in the TCD group than in the non-TCD group (62% v 24%, p = .0003). After donor lymphocyte infusion (DLI), however, 17 of 20 patients in the TCD group and 2 of 3 patients in the non-TCD group achieved a complete remission. While the CML experience clearly supports the critical role of GVL in allogeneic HCT, results in studies with patients with AML or ALL report comparable low rates of relapse after TCD HCT.[11, 12, 13, 14, 17, 18] For example, in the BMT CTN 0303 study, the relapse rate for patients with AML in CR1 was 17.4% at 36 months.[14] The MSKCC group recently reported results in 56 adult patients with ALL, including 27 patients in CR1, 18 in CR2, 11 in CR3 or greater.[17] With a median follow-up of 6.1 years, the cumulative incidence of relapse for the entire cohort was 23%. These results are consistent with those reported by the Perugia group who found a probability of relapse of 0.12 for patients with AML and 0.28 for patients with ALL who underwent TCD HCT in CR1 or CR2.[25] To further assess the impact of T-cell depletion on relapse in patients with AML in CR1, a retrospective analysis of 115 patients who received TCD grafts after ablative conditioning at MSKCC were compared to a cohort of 181 patients who received unmodified grafts after conditioning with busulfan/ fludarabine and GVHD prophylaxis with tacrolimus/ mini-methotrexate at MD Anderson Cancer Center (MDACC).[18] There were no significant differences in the rate of relapse at 3 years between TCD and unmodified graft recipients (18% vs. 25%, p= 0.3).

1.2.3. Immune Reconstitution after CD34 Selection

Allogeneic HCT is associated with deficiencies in the recovery of T and B cells that are associated with increased rates of infections,[30, 31, 32, 33] disease relapse,[34] and the development of secondary malignancies.[35] While several factors contribute to these immune defects, the use of either *in vivo* (with alemtuzumab or ATG) or *ex vivo* T-cell depletion has marked effects on immune recovery.[6, 7, 8, 9, 30, 36, 37, 38, 39, 40, 41] Studies of thymic output have shown lower T-cell receptor rearrangement excision circles (TRECs) in recipients of TCD allografts compared to unmodified allograft recipients.[41] These differences, however, abated beyond 9 months. Delayed thymic output translates into delayed recovery of total and naïve CD4+ T-cells, prolonged inversion of the CD4/CD8 ratio, and delayed recovery of T-cell mitogen responses, which is associated with an increase in Epstein-Barr Virus-Associated Lymphoproliferative disorders and opportunistic infections in the first year post-transplant.[30, 40] Studies of T-cell receptor (TCR) diversity in allo-HCT patients using 5'-RACE PCR with deep sequencing have shown more rapid recovery in diversity in recipients of conventional grafts compared to TCD grafts.[42] It should be noted, however, that GVHD also has a significant impact on immune recovery through direct effects on the thymus,[43, 44] and particularly because of the immunosuppressive drugs required to treat GVHD.[45, 46, 47, 48, 49] In the MSKCC/MDACC retrospective study, 6 (5%) and 2 (1%) patients died of infections within 100 days post-transplant, in the TCD and unmodified groups (p=0.04), respectively.[18] Despite these differences in infectious deaths, there were no significant differences in three-year relapse-free (RFS) and overall survival (OS) rates.

1.2.4. Advantages of CD34- Selection

The main advantage of the use of a CD34-selected graft is the significant reduction in acute and chronic GVHD. This is particularly relevant in patients who do not have a fully matched donor. Another advantage of CD34-selected grafts is the fact that no post-transplant GVHD prophylaxis is required. The ability to avoid CNI eliminates the renal toxicity associated with their use in allo-HCT therefore allows inclusion of patients with underlying renal dysfunction, including older patients. The lack of CNI increases the ability to use ablative regimens in older patients where the combined toxicity of an ablative regimen and post-transplant GVHD prophylaxis that includes a CNI and methotrexate represents a dose limiting toxicity. As a result, older patients receiving conventional grafts are typically treated with reduced intensity or non-ablative conditioning regimens, which are associated with higher rates of relapse in acute leukemias. Finally, the use of a CD34-selected graft also represents the ideal platform for post-transplant immunotherapy with adoptive cell therapy targeting minimal residual disease. This approach has the potential advantage of overcoming any loss of GVL without affecting the benefit of reduced GVHD.

T-cell depletion has now been under investigation for over 3 decades with few studies comparing outcomes with patients receiving unmodified grafts. As noted above, results from the MSKCC/MDACC comparative study demonstrated similar RFS and OS after TCD and conventional transplants from related/unrelated donors in patients with AML in CR1, but a significant reduction in GVHD in the TCD cohort.[18] A prior retrospective study of 146 patients with hematological malignancies did not show significant differences in survival, GVHD rates, and quality of life between patients who received TCD and unmodified grafts, although the method of T-cell depletion used only achieved a 1-2 log reduction in T-cells.[50] In the only prospective randomized phase II-III trial, the incidence of acute GVHD was lower after TCD-HCT, but there was no difference in survival.[51] This study also used older methods of T-cell depletion that only achieved 1 - 2 logs of depletion. A recent analysis of the BMT CTN 0303 patients compared to a subset of patients on BMT CTN 0101 who received a conventional transplant for AML in CR1/CR2 showed no differences in rates of graft rejection, leukemia relapse, transplant-related mortality (TRM), and disease-free and overall survival rates.[10] Two-year rates of chronic GVHD were lower with TCD grafts than conventional grafts (19% v 50%, respectively; P < .001) and TCD was associated with a higher GVHD-free survival at 2 years (41% v 19%; P = .006).

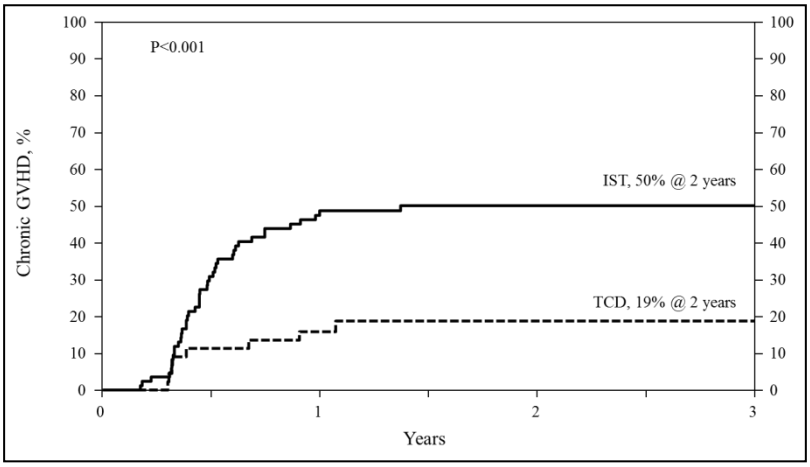


Figure 1.2: Chronic GVHD comparing recipients of T-cell depleted graphs (TCD) to recipients of unmanipulated grafts with chronic immunosuppression (IST) (Pasquini MC et al, 2012 JCO)

1.3. Post-Transplant Cyclophosphamide

1.3.1. Rationale of Post-Transplant Cyclophosphamide

High-dose cyclophosphamide is a potent immunomodulatory agent that has been successfully used to prevent GVHD in HLA-matched and –mismatched setting in several single-center as well as in multi-center studies. Preclinical studies have shown that cyclophosphamide administered early post HSCT preferentially kills activated, cycling allo-reactive T-cells while sparing resting, non allo-reactive T-cells leading to suppression of GVHD as well as graft rejection.[52, 53] Furthermore, a recent study showed that human regulatory T cells are resistant to post-transplant cyclophosphamide (PTCy) and contribute to its GVHD preventive effects.[54]

1.3.2. Post-Transplant Cyclophosphamide in Haploidentical Donor Transplants

Based on promising pre-clinical results at Johns Hopkins, a Phase I/II clinical trial of haploidentical HCT to treat high-risk hematologic malignancies was initiated in 1999.[52, 53] Following a non-myeloablative regimen of fludarabine, cyclophosphamide, and low-dose TBI, GVHD prophylaxis consisted of cyclophosphamide (Cy) given on Days +3 and +4 post-transplant, tacrolimus, and mycophenolate mofetil (MMF).[41] Primary graft failure occurred in 13% of patients, and was fatal due to infection in one patient in whom autologous hematopoiesis failed to occur. In general, complete T-cell engraftment was observed by Day +28 or the grafts were rejected. Cumulative incidences of grades II-IV and grades III-IV acute GVHD by Day 200 were 34% and 6%, respectively. There was lower incidence of extensive chronic GVHD among recipients of two versus one dose of post-transplantation Cy (5% versus 25%; p=0.05). There was no difference in the incidence of severe acute GVHD with one or two doses of post-transplant Cy. The cumulative incidences of non-relapse mortality and relapse at 1 year were 15% and 51%, respectively. Actuarial overall and event-free survivals (EFS) at two years after transplantation were 36% and 26%, respectively. Patients with lymphoid malignancies appeared to have an improved EFS compared to those with myeloid malignancies (p=0.02).

The BMT CTN sponsored a multi-center Phase II trial of haploidentical BMT (BMT CTN 0603) for high-risk hematologic malignancies modeled after the Hopkins approach. This was published along with a similar study using double umbilical cord blood (dUCB) grafts without post-transplant Cy (BMT CTN 0604).[39] The 1-year probabilities of overall and progression-free survival were 54% and 46% after dUCB transplantation and 62% and 48% after haploidentical bone marrow HCT. The Day +56 cumulative incidence of neutrophil recovery was 94% after dUCB and 96% after haploidentical marrow.

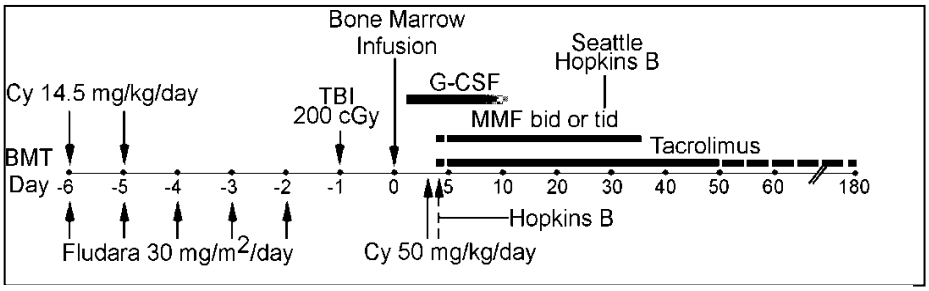


Figure 1.3.2: Treatment schema of patients undergoing haploidentical donor transplants in the BMT CTN 0603 clinical trial.

The 100-day cumulative incidence of grade II-IV acute GVHD was 40% with dUCB and 32% with haploidentical bone marrow. The 1-year cumulative incidences of non-relapse mortality and relapse after cord transplantation were 24% and 31%, respectively; corresponding rates after haploidentical bone marrow transplantation were 7% and 45%.

Several other centers have reported studies using PTCy to facilitate haploidentical transplantation in the myeloablative conditioning setting.[55 56 57 58] The outcomes reported with these studies are consistent with the low NRM seen in studies of non-myeloablative T-cell replete haploidentical donor transplantation performed using PTCy as well as consistently low rates of severe acute and chronic GVHD.

To date, haploidentical non-myeloablative transplantation with post-transplant Cy has used bone marrow as the graft source. Use of PBSC instead of marrow may allow wider applicability of this approach but there is concern about higher risks of acute and chronic GVHD due to the 5-10-fold higher number of T-cells in the allograft. Recently, groups in Atlanta and Seattle/London reported small studies in which PBSC were substituted for bone marrow with post-transplant Cy in the haploidentical donor setting.[59 60] In both studies, PBSC was used for HLA-haploidentical HCT with PTCy with overall outcomes of severe acute GVHD, chronic GVHD, and non-relapse mortality at 1 year with HLA-matched related or unrelated HCT.

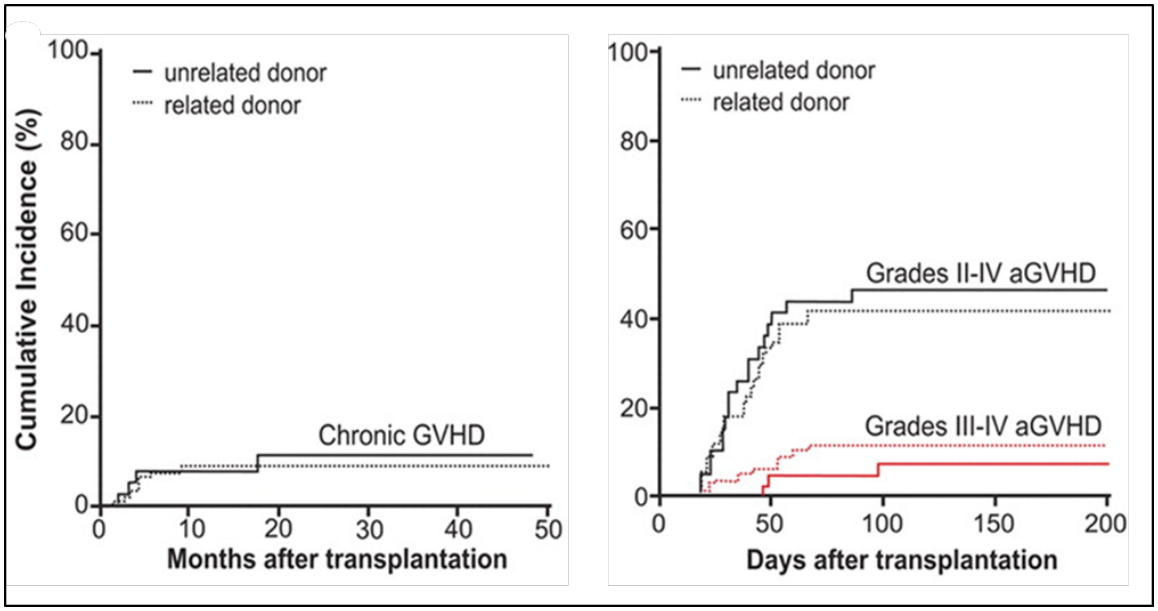
A number of studies accompanying clinical trials of haploidentical HCT with post-transplant Cy have been performed. Kasamon et al. looked at the effect of HLA disparity on transplant outcomes in a retrospective analysis of 185 patients transplanted in Baltimore.[61] No association was found between the degree of mismatching at 5 HLA loci and the risk of acute GvHD or NRM. A retrospective study by Burroughs, et al.,[62] compared the outcomes of patients with relapsed Hodgkin lymphoma (92% of whom had failed autologous transplants) who had undergone nonablative conditioning and transplantation with either haploidentical donors or HLA-matched related or unrelated donors in Baltimore or Seattle. GvHD prophylaxis after transplantation with matched donors was a calcineurin inhibitor plus MMF. Although the number of patients in each group was relatively small, it appeared that outcomes after haploidentical transplantation were no worse and possibly better than those after transplantation from matched donors. Two year NRM was similar for haploidentical and unrelated donors (9% and 8%, respectively) compared to 21% for related donors. EFS at 2 years was 51% for haploidentical donors compared to 23% and 29% for related and unrelated donors, respectively. The finding of similar outcomes after haploidentical HCT using PTCy compared with matched related or unrelated donors for a variety of hematologic malignancies was also recently reported.[63]

1.3.3. PTCy in HLA Matched Donor

Given the success of PTCy in the prevention of GVHD in the setting of haploidentical transplantation, Luznik et al.[64] reported a large study of patients who underwent allogeneic HCT from HLA-matched donors after myeloablative conditioning using busulfan-cyclophosphamide (BuCy) and PTCy as a single agent for prophylaxis of GvHD. A total of 117 patients with high-risk hematologic malignancies were transplanted from 78 related or 39 unrelated donors. Half of the patients were not in remission at the time of transplant. Bone marrow grafts were used for all

patients. The incidence of GVHD was remarkably low (43% grades II-IV acute, 10% grades III/IV acute, 10% chronic) showing the effectiveness of the approach (Figure 1.3.2). There was no difference in the incidence of acute or chronic GVHD between related or unrelated donors. Almost two-thirds of the patients did not require any additional immunosuppressive therapy after post-transplant Cy. Patients who did develop GVHD responded to steroids alone in 20% of cases or steroids plus a second agent (calcineurin or non-calcineurin) in 75% of cases. Rates of grade II-IV acute and chronic GVHD were both 10% (Figure 1.3.2). Observed rates of non-relapse mortality and disease relapse were 20% and 44%, respectively. This study showed that post-transplant Cy was effective as a single-agent to prevent severe acute or chronic GVHD in the vast majority of patients undergoing myeloablative transplantation from matched donors. Only 3% of deaths were due to infection suggesting that immune reconstitution is robust in such patients. Obviating the need for ongoing immunosuppression post-transplant provides an optimal platform for cellular therapy to prevent or treat relapsed disease.

These encouraging outcomes with PTCy in prevention of GVHD were recently reproduced in a multi-institutional study that effectively combined this novel single-agent short-course GVHD prevention strategy in combination with IV Bu/Flu myeloablative conditioning. In this study, 92 adult patients (median age 49, range 21-65) with high-risk hematologic malignancies were enrolled at three centers. Forty-five (49%) patients received related allografts, and 47 (51%) received unrelated allografts. GVHD prophylaxis was solely with PTCy at 50 mg/kg/day on post-transplant days +3 and +4 after bone marrow allografting. The cumulative incidences of grade II-IV acute, grade III-IV acute, and chronic GVHD were 51%, 15%, and 14%, respectively. Non-relapse mortality (NRM) at 100 days and 1 year were 9% and 16%, respectively. With a 2.2 year median follow-up, the two-year event-free survival (EFS) and overall survival (OS) were 62% and 67%, respectively. Donor type did not impact on NRM, EFS, or OS. Patients in complete remission (CR) without evidence of minimal residual disease had remarkably high rates of EFS (80%) and OS (79%). The results obtained in these two studies serve as the basis for the PTCy CNI-free strategy in this clinical trial.



1.3.4. Immune Reconstitution after PTCy

The ability to shorten the duration of post-grafting immunosuppression after HLA-matched allo-HSCT with PTCy was marked by prompt immune reconstitution and a low incidence of opportunistic infections. Among 47 patients studied immunophenotypically after HLA-matched HCT the mean $CD4^+$ T cell count on day 30 was 98 cells µl and on day 60 was 124 cells/µl.[54] At both days 30 and 60 after HCT, the $CD8^+$ T cell counts were already within the normal range. Effector regulatory T cells rapidly recovered to donor levels by 30 days after HCT, and there was favorable memory $CD4^+$ T cell recovery compared with naïve $CD4^+$ T cells.

In both studies detailed in section 1.3.3. above, no patients died of CMV or invasive fungal infection. Reactivation of CMV occurred in 29% of patients, and there were only two documented cases of CMV disease. The rapid recovery of CMV-specific immunity correlated with the results of *in vitro* ELISPOT assays. The frequency of cells secreting interferon gamma in response to stimulation with pentadecapeptides of the immunodominant CMV protein, pp65, at day 30–60 after allo-HSCT did not differ from pre-transplantation specimens from CMV-seropositive donor/recipient pairs. The absence of post-transplantation Epstein–Barr virus-associated lymphoproliferative disease is another indicator of the prompt immunologic recovery seen with post-transplant Cy. [65]

1.4. Benchmark Analysis and Composite Endpoint

In order to better evaluate the efficacy of novel approaches for GVHD prophylaxis, a benchmark analysis was performed using data from the Center for International Blood and Marrow Transplant Research (CIBMTR) for patients who received standard CNI based GVHD prophylaxis with tacrolimus and MTX.

The CIBMTR maintains an outcomes registry that prospectively collects data from all centers performing allogeneic HCTs and almost all centers performing autologous HCTs in the United States in addition to about 100 non-US centers. Centers must report all consecutive patients and provide longitudinal follow-up on those patients according to set timelines that include a pre-transplant report, a 100 day report, a 6 month report and an annual report through 6 years post-transplant followed by a biannual report in perpetuity. Data are reported on two tracks: a "Transplant Essential Data" track and a "Comprehensive Report Form" track. Centers provide a pre-transplant Transplant Essential Data form for all patients. Data from this form are used to select patients for the Comprehensive Report Form track using a weighted random selection that over selects patients with rare diseases or procedures or for the purposes of specific studies. For example, most patients on BMT CTN trials are selected for the Comprehensive Report Form track so that data collected by the CIBMTR can supplement clinical trial data collected through AdvantageEDC^{SM} and can allow for long-term follow-up of trial patients for specific late effects of treatment. Longitudinal data are collected for patients on both the Transplant Essential Data and Comprehensive Report Form Track; the data differ in quantity and granularity. Data quality is ensured by computerized error checks and on-site audits.

The objective of the benchmark analysis was to select promising approaches to be further studied and to explore novel endpoints that could not only assess GVHD, but also the complex relationships between relapse and GVHD as well as prolonged use of immune suppression. The control population selected from the CIBMTR database was comprised of patients who received HSCT in a US center from 2006 to 2009 and who received tacrolimus and methotrexate as their sole GVHD prophylaxis. Data from single institution studies of the three agents to be tested in this protocol were also studied. Populations differed according to disease, donor, conditioning intensity, disease risk and patient age. Six investigational approaches were evaluated: tacrolimus/MTX+etanercept, tacrolimus/MTX+pentostatin/ATG, PTCy, CD34-selection, tacrolimus/MTX+bortezomib and tacrolimus/MTX+maraviroc and compared to CIBMTR controls. Each investigational cohort differed in respect to size, disease status, graft source, conditioning regimen intensity and donor type. These differences between the investigational and control cohorts were adjusted using a Cox Regression models analyzing GVHD and survival outcomes (**Table 1.4**). CD34-selection showed promising results in GVHD and survival outcomes, and PTCy showed superiority in chronic GVHD outcomes compared to controls.

Table 1.4: RESULTS FROM THE CIBMTR BENCHMARK ANALYSIS OF GVHD PROPHYLAXIS REGIMENS

Outcome	Time[1]/ HR[2]	Tacro/MTX N=5,048	Etanercept N=74	Pentostatin/ATG N=66	PTCy N=117	CD34-sel N=291	Bortezomib N=44	Maraviroc N=33
Acute Grade 3-4 GVHD	100d (%) 95% CI	23 (22-24)	21 (14-31)	14 (7-23)	21 (13-30)	4 (2-8)	10 (4-20)	4 (0-18)
	HR 95% CI	1.00	0.86 (0.5-1.3)	**0.50* (0.3-0.9)**	0.90 (0.6-1.4)	**0.31* (0.2-0.5)**	**0.48* (0.2-1.0)**	0.91* (0.4-2.0)
Chronic GVHD	12 m (%) 95% CI	45 43-46	59 (47-69)	39 (27-51)	13 (7-20)	8 (5-11)	43 (28-58)	19 (7-35)
	HR 95% CI	1.00	1.50* (1.1-2)	**0.60* (0.4-0.9)**	**0.24* (0.1-0.4)**	**0.10* (0.1-0.2)**	0.73 (0.5-1.1)	0.29 (0.1-0.7)
Overall Survival	12 m (%) 95% CI	60 58-61	66 (55-75)	63 (52-73)	57 (47-66)	73 (67-78)	79 (66-88)	64 (47-77)
	HR 95% CI	1.00	0.85 (0.6-1.2)	**0.69* (0.5-0.9)**	1.07 (0.8-1.4)	**0.68* (0.5-0.8)**	**0.53* (0.3-0.8)**	0.80 (0.5-1.4)

In addition to the standard variables of GVHD and survival, we studied a composite outcome that combined three important outcomes with median times of occurrence >90 days after HCT: chronic GVHD, relapse and all-cause mortality (CRFS). The proportion of patients not achieving this composite outcome within the CIBMTR controls was 20% by one year post-transplant (**Figure 1.4**). Thus, with current standard transplant approaches, only one fifth of patients are alive, disease- and GVHD-free at one year postHCT. The BMT CTN Steering Committee felt that improvement on this dismal result should be the focus of the next generation of transplant trials and a goal for improvement in the field.

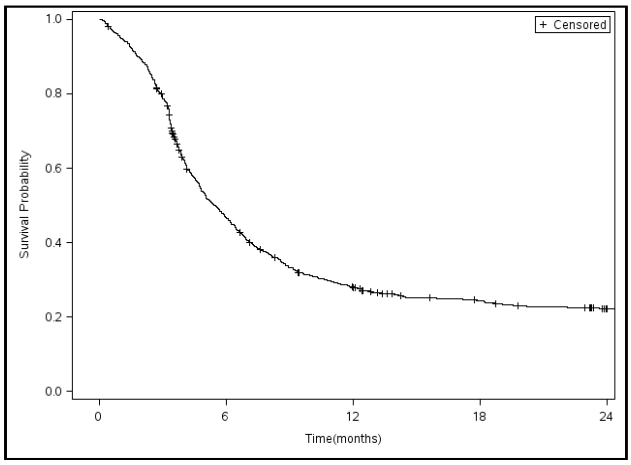


Figure 1.4: Chronic GVHD/Relapse-free survival (CRFS) after bone marrow transplantation using calcineurin inhibitor based GVHD prophylaxis.

1.5. Rationale

This multicenter Phase III clinical trial will evaluate two CNI-free approaches for their efficacy in improving the proportion of patients who do not develop moderate to severe chronic GVHD, disease progression or relapse or death compared to recipients of CNI-based GVHD prophylaxis. The results of this study will inform whether these CNI-free approaches offer advantage over CNI based according to this novel CRFS without the need for chronic immunosuppression.

CHAPTER 2

2. STUDY DESIGN

2.1. Study Overview

This is a Phase III randomized, open label, multicenter trial comparing two CNI-free strategies: CD34 selection in PBSC grafts and infusion of BM grafts followed by high dose cyclophosphamide (PTCy) to unmanipulated BM transplants with standard CNI (tacrolimus) and methotrexate (Tac/MTX) GVHD prophylaxis in patients with hematologic malignancies undergoing myeloablative conditioning hematopoietic cell transplantation. The primary endpoint of chronic GVHD/relapse-free survival (CRFS) as a time to event endpoint will be compared between the two CNI-free strategies as well as between each CNI-free strategy and the CNI-based controls.

2.2. Hypothesis and Specific Objectives

2.2.1. Hypothesis

CNI-free approaches result in lower rates of chronic GVHD and relapse and improved survival compared to standard CNI-based GVHD prophylaxis.

2.2.2. Study Objectives

The primary objective of the randomized trial is to compare CRFS after hematopoietic stem cell transplant (HSCT) across the three arms of this trial. Secondary objectives are to compare rates of grade II-IV and III-IV acute GVHD and chronic GVHD; chronic GVHD-free survival; immunosuppression-free survival; neutrophil and platelet engraftment; disease relapse; transplant-related mortality; rates of Grade ≥ 3 toxicity according to the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0; specifically rates of hepatic sinusoidal obstructive syndrome (SOS) and idiopathic pneumonia syndrome (IPS), incidence of infections; specifically infections by CMV and EBV, quality of life and overall survival across the three arms of this trial.

2.3. Patient Eligibility

2.3.1. Inclusion Criteria

1. Males and females aged ≥ 1.0 year and < 66.0 years
2. Patients with acute leukemia in morphologic complete remission with or without hematologic recovery or with myelodysplasia (MDS) with no circulating blasts and with less than 5% blasts in the bone marrow. Patients with CMML must have a WBC count ≤ 10,000 cells/µL and < 5% blasts in the marrow. Patients with ≥ 5% blasts due to a regenerating marrow must contact the protocol chairs for review.
3. Planned myeloablative conditioning regimen (see eligible regimens in Table 2.4)

4. **Patients must have a related or unrelated donor as follows:**
    a. Related donor must be an 8/8 match for HLA-A, -B, and -C at intermediate (or higher) resolution, and -DRB1 at high resolution using DNA-based typing. Pediatric related donors must weigh ≥ 25.0 kg., must have adequate peripheral venous catheter access for leukapheresis or must agree to placement of a central catheter, must be willing to (1) donate bone marrow and (2) receive G-CSF followed by donation of peripheral blood stem cells (product to be determined by randomization post enrollment) and must meet institutional criteria for donation[1].
    b. Unrelated donor must be an 8/8 match at HLA-A, -B, -C and –DRB1 at high resolution using DNA-based typing. Unrelated donor must be medically eligible to donate according to NMDP (or equivalent donor search organization) criteria. At time of enrollment, the donor should not have any known preferences or contraindications to donate bone marrow or peripheral blood stem cells[1].
        i. Selection of unrelated donors is to be performed according to institutional practice. It is recommended that the time from collection to initiation of the cell processing be considered when prioritizing donors, as data shows better results for CD34 selection when cell processing begins within 36 hours of the end of collection as indicated in section 2.5.1.2.
5. Cardiac function: Ejection fraction at rest ≥ 45.0% or shortening fraction of ≥ 27.0% by echocardiogram or radionuclide scan (MUGA**).**
6. Estimated creatinine clearance (for patients > 12 years) greater than 50.0 mL/minute (using the Cockcroft-Gault formula and actual body weight); for pediatric patients (≥ 1 year to 12 years), GFR estimated by the updated Schwartz formula ≥ 90.0 mL/min/1.73 m^2. If the estimated creatinine clearance is < 90 mL/min/1.73 m^2, then renal function must be measured by 24-hour creatinine clearance or nuclear GFR, and must be > 70.0 mL/min/1.73 m^2.
7. Pulmonary function: DLCO ≥ 50% (adjusted for hemoglobin), and FVC and FEV1 ≥ 50%; for children who are unable to perform for PFTs due to age or developmental ability, there must be no evidence of dyspnea and no need for supplemental oxygen, as evidenced by O_2 saturation ≥ 92% on room air.
8. Liver function: total bilirubin < 2x the upper limit of normal (unless elevated bilirubin is attributed to Gilbert's Syndrome) and ALT/AST < 2.5x the upper limit of normal.
9. Signed informed consent.

2.3.2. Exclusion Criteria

1. Prior autologous or allogeneic hematopoietic stem cell transplant

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[1] For unrelated donors, during the period of confirmatory typing, some donors may have preferences or contraindications to donate a certain stem cell graft source. Similar information would apply to related donors during the donor work up procedures. If a patient has only one potential donor and the donor has restrictions on which product he/she will donate, then the patient would not be eligible for this study.

2. Karnofsky or Lansky Performance Score < 70%
3. Active CNS involvement by malignant cells
4. Patients with uncontrolled bacterial, viral or fungal infections (currently taking medication and with progression or no clinical improvement) at time of enrollment
5. Presence of fluid collection (ascites, pleural or pericardial effusion) that interferes with methotrexate clearance or makes methotrexate use contraindicated
6. Patients seropositive for HIV-1 or -2
7. Patients seropositive for HTLV-I or -II
8. Patients with active Hepatitis B or C viral replication by PCR
9. Documented allergy to iron dextran or murine proteins
10. Women who are pregnant (positive serum or urine βHCG) or breastfeeding
11. Females of childbearing potential (FCBP)[2] or men who have sexual contact with FCBP unwilling to use 2 effective forms of birth control or abstinence for one year after transplantation
12. History of uncontrolled autoimmune disease or on active treatment
13. Patients with prior malignancies, except resected non-melanoma or treated cervical carcinoma in situ. Cancer treated with curative intent ≥ 5 years previously will be allowed. Cancer treated with curative intent < 5 years previously will not be allowed unless approved by the Protocol Officer or one of the Protocol Chairs.
14. Patient unable to comply with the treatment protocol including appropriate supportive care, follow-up and research tests
15. Planned post-transplant maintenance therapy except for FLT3 inhibitors or TKIs
    a. Must be declared prior to randomization.
16. If it is known prior to enrollment that the hematopoietic stem cell product will need to be cryopreserved, **the patient should not be enrolled**.
17. *German centers only*: Treatment with any known non-marketed drug substance or experimental therapy within 5 terminal half lives or 4 weeks prior to enrollment, whichever is longer, or participation in any other interventional clinical study.

[2] A female of childbearing potential is a sexually mature female who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

2.4. Conditioning Regimens

Minor modifications to the myeloablative regimens that involve start day or days of rest in the Control Arm are allowed but must be approved by the protocol chairs. Only minor modifications will be considered for approval. Modifications to the myeloablative regimens in the intervention arms (CD34 Cell Selection Arm and Post-Transplant Cy Arm) are not allowed. An additional day of rest may be added to conditioning regimens of any arm when transportation and/or delivery of the patient's graft is delayed.

For patients who weigh less than 125% of their ideal body weight (IBW), dosing should be based on actual body weight. These dosing rules apply to all chemotherapy drugs listed in section 2.4. including cyclophosphamide, thiotepa, rATG, busulfan, fludarabine, melphalan and etoposide. Ideal and actual body weight units are in kg.

Patients ≥ 18.0 Years of Age
Males IBW = 50 kg + 2.3 kg/inch over 5 feet (60 in)
Females IBW = 45.5 kg + 2.3 kg/inch over 5 feet (60 in)
For patients less than 5 feet, subtract 2.3 kg/inch

Metric Conversion
Males IBW = 50 kg + 2.3 kg ([height in meters -1.52] /0.025)
Females IBW = 45.5 kg + 2.3 kg ([height in meters -1.52] /0.025)
For patients shorter than 1.52 m, subtract 2.3 kg/ for each 2.5 cm below 1.52m

Patients ≥ 1.0 Year and < 18.0 Years of Age

Height Less than 60 inches (5 feet or 1.52m)
IBW = (ht^2 x 1.65)/1000 where ht = cm, IBW = kg

Height More than 60 inches (5 feet or 1.52m)
Males IBW = 39.0 + [2.27 x (ht - 60)] where ht = inches, IBW = kg
Females IBW = 42.2 + [2.27 x (ht - 60)] where ht = inches, IBW = kg

Metric Conversion
Males IBW = 39.0 + [2.27 x (height in meters – 1.52/0.025)]
Females IBW = 42.2 + [2.27 x (height in meters– 1.52/0.025)]

For patients who weigh greater than or equal to 125% of their IBW, dosing should be based on the adjusted ideal body weight (AIBW).

Adjusted Ideal Body Weight (AIBW) Formula:

AIBW = IBW + [(0.25) x (actual body weight – IBW)]

TABLE 2.4: ALLOWABLE MYELOABLATIVE REGIMENS BY TREATMENT ARM

CD34 Selection Arm (PBSC)	
§ 2.4.1.	**Total Body Irradiation/ Cyclophosphamide/Thiotepa/rATG** • TBI (1320 – 1400 cGy) • Cyclophosphamide (120 mg/kg) • Thiotepa (10 mg/kg) • rATG (5mg/kg)
§ 2.4.2.	**Busulfan/Melphalan/Fludarabine/rATG** • Busulfan (9.6 mg/kg IV) • Fludarabine (125 mg/m^2) • Melphalan (140 mg/m^2) • rATG (5mg/kg)

Post-Transplant Cy Arm (BM)			Control Arm (BM)
§ 2.4.3.1.	**Busulfan[a]/Cyclophosphamide (Bu/Cy)** • Busulfan (16 mg/kg PO or 12.8 mg/kg IV) • Cyclophosphamide (100 mg/kg)	**§ 2.4.3.2.**	**Busulfan[a]/Cyclophosphamide (Bu/Cy)** • Busulfan (16 mg/kg PO or 12.8 mg/kg IV) • Cyclophosphamide (120 mg/kg)
§ 2.4.4.	**Busulfan[a] /Fludarabine (Bu/Flu)** • Busulfan (16 mg/kg PO or 12.8 mg/kg IV) • Fludarabine (160 mg/m^2)	**§ 2.4.4.**	**Busulfan[a] /Fludarabine (Bu/Flu)** • Busulfan (16 mg/kg PO or 12.8 mg/kg IV) • Fludarabine (160 mg/m^2)
§ 2.4.5.1.	**Cyclophosphamide/Total Body Irradiation (Cy/TBI)** • Cyclophosphamide (100 mg/kg) • TBI (1200-1420 cGy)	**§ 2.4.5.2.**	**Cyclophosphamide/Total Body Irradiation (Cy/TBI)** • Cyclophosphamide (120 mg/kg) • TBI (1200-1420 cGy)
		§ 2.4.6.	**Total Body Irradiation/Etoposide (TBI/Etoposide)** • TBI (1200-1320 cGy) • Etoposide (60 mg/kg)

[a] Bu = PO doses will be adjusted to maintain BU Css at 900±100 ng/ml.

2.4.1. TBI, Cyclophosphamide, Thiotepa, and rATG (Cy/TBI/Thio/rATG)

Table 2.4.1. - CD34 Cell Selection Arm: Total Body Irradiation/ Cyclophosphamide/ Thiotepa/ rATG Regimen

Days -9 to -6	TBI (1375cGy): Administered at a dose rate of < 20 cGy/minute. Doses of 125 cGy/fraction are administered at a minimum interval of 4 hours between fractions, three times/day for a total of 11 doses (1375 cGy) over 4 days
Days -5 to -4	Thiotepa 5 mg/kg/day: IV for two consecutive days
Days -4 to -3	rATG 2.5 mg/kg/day: Given as a single intravenous dose on Day -4 and Day -3 using rabbit antithymocyte globulin (Genzyme)
Days -3 to -2	Cyclophosphamide 60 mg/kg/day: IV for two consecutive days

Hyperfractionated TBI is administered at a dose rate of < 20 cGy/minute. Doses of 125 cGy/fraction are administered at a minimum interval of 4 hours between fractions, three times/day for a total of 11 doses (1375 cGy) over 4 days (Day -9, -8, -7 and -6). Sequential doses are administered in an anterior/posterior or lateral orientation. The orientation of TBI chosen will be left to the discretion of the radiation oncology specialist at each center but should remain consistent at each institution throughout the duration of the trial. Full value lung blocks are not allowed. Compensators and lung blocks yielding a minimum of 800 cGy lung dose are allowed based on institutional practice. Depending on the method of lung shielding employed and institutional practice, the blocked areas should be boosted with high-energy electrons or be otherwise radiated so that the cumulative chest wall dose is approximately 1300 cGy, so as to insure that marrow sites in the ribs are adequately treated. Palifermin may be given to patients receiving TBI per institutional practices.

In addition, male patients receiving transplants for ALL or AML, use of boost to testes is allowed according to institutional practices.

If general anesthesia is required for TBI administration (e.g., young children), a dose of 200cGy q12h x 7 doses to a total dose of 1400 cGy may be given. Alternatively, a dose of 165 cGy q12h x 8 doses to a total dose of 1320 cGy may be given to patients < 18 years according to institutional guidelines.

Thiotepa will be administered at a dose of 5mg/kg/day IV for two consecutive days (Day -5 and -4).

Rabbit anti-thymocyte globulin (Thymoglobulin®) will be given as a single intravenous dose on Day -4 and Day -3 using rabbit antithymocyte globulin (Genzyme) at a dose of 2.5 mg/kg/day. If the patient has a history of allergy or intolerance to rabbit ATG, equine antithymocyte globulin at a dose of 30 mg/kg may be used. The dose of ATG will be administered over 6-8 hours. Methylprednisolone 1 mg/kg will be given as premedication x 2 days with the ATG administration

and will be discontinued thereafter. Additional medications to prevent or treat reactions will be administered as indicated according to institutional guidelines**.** If severe reaction is encountered after the first dose of ATG, the second dose can be delayed until Day +5.

Cyclophosphamide will be administered at a dose of 60 mg/kg/day IV for two consecutive days (Day -3 and -2). The Day –2 dose should be given in the morning, preferably before 10AM. Use of Mesna and dosing will be done according to institutional standards. A recommended approach is as follows: Mesna dose of ≥ 80% of the total daily dose of Cy and given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy intravenously.

2.4.2. Busulfan, Melphalan, Fludarabine, and rATG

Table 2.4.2. – CD34 Cell Selection Arm: Busulfan, Melphalan, Fludarabine, and rATG Regimen

Days -9 to -7	Busulfan: if >4 years old: 0.8 mg/kg/dose Q6h IV if ≤ 4 years old:1 mg/kg/dose Q6h IV Infused over two hours.
Days -6 to -5	Melphalan: if weight > 12 kg: 70 mg/m^2/day if weight ≤ 12 kg: 2.3 mg/kg/day Infused once daily intravenously over 30 minutes
Days -6 to -2	Fludarabine 25 mg/m^2/day: Administered intravenously over 30 minutes at a total dose of 125 mg/m^2 divided into 5 daily doses of 25 mg/m^2/day
Days -3 to -2	rATG 2.5 mg/kg/day: Will be given at 2.5 mg/Kg/day x 2 days on Days -3 and -2

Busulfan will be infused over 2 hours. Phamacokinetics on busulfan based on test dose or the first dose can be done according to institutional practices. Busulfan pharmacokinetics studies are strongly encouraged with first AUC of 800-1400 μMol-min or Bu Css 900 ± 100 ng/mL/, but are not mandated by this protocol.

Melphalan will be infused once daily intravenously over 30 minutes.

Fludarabine will be administered intravenously over 30 minutes at a total dose of 125 mg/m^2 divided into 5 daily doses of 25mg/m^2/day.

Rabbit anti-thymocyte globulin (Thymoglobulin®) will be given at 2.5 mg/Kg/day x 2 days on Days -3 and -2. If the patient has a history of allergy or intolerance to rabbit ATG, equine antithymocyte globulin at a dose of 15 mg/kg/day x 2 or 30 mg/kg x 1 may be used. The dose of

ATG will be administered over 6-8 hours. Methylprednisolone 1 mg/kg will be given as premedication x 2 days with the ATG administration and will be discontinued thereafter. Additional medications to prevent or treat reactions will be administered as indicated according to institutional guidelines**.** If severe reaction is encountered after the first dose of ATG, the second dose can be delayed until Day +5.

2.4.3. Busulfan and cyclophosphamide (Bu/Cy)

2.4.3.1. The allowable Bu/Cy regimen for the Post-Transplant Cy Arm is the following:

Table 2.4.3.1. – Post-Transplant Cy Arm: Busulfan and Cyclophosphamide Regimen

Days -7 to -4	Busulfan*: 4 mg/kg/day PO, total dose of 16 mg/kg PO 3.2 mg/kg/day IV total dose of 12.8 mg/kg
Days -3 to -2	Cyclophosphamide: 50 mg/kg/day, total dose of 100 mg/kg

*Busulfan should be administered per institutional guidelines but dosing divided in q6h administration x 4 days is recommended for patients randomized to the PTCy treatment arm.

2.4.3.2. The allowable Bu/Cy regimen for the Control Arm is the following:

Table 2.4.3.2. – Control Arm: Busulfan and Cyclophosphamide Regimen

Days -7 to -4	Busulfan: 4 mg/kg/day PO, total dose of 16mg/kg PO 3.2 mg/kg/day IV total dose of 12.8 mg/kg
Days -3 to -2	Cyclophosphamide: 60 mg/kg/day, total dose of 120 mg/kg

Participating centers will have the option of using oral or intravenous Busulfan (Bu). **Oral Bu** will be administered at 4 mg/kg/day on Days -7, -6, -5 and -4 (1mg/kg every 6 hours). If the center is administering busulfan orally, pharmacokinetics analysis and targeting the dose to 900±100 ng/mL must be performed or first AUC of 800-1400 Bu Css 900 ± 100 ng/mL/or AUC of 800-1400 μMol-min.

Intravenous Bu is administered at a dose of 3.2 mg/kg/day or 130 mg/m^2/day on Days -7, -6, -5 and -4 either in four divided doses (0.8 mg/kg) or once daily (3.2 mg/kg or 130 mg/m^2). Patients randomized to the PTCy arm, it is recommended that Bu dosing be divided in a Q6h schedule for four days. Busulfan pharmacokinetics studies are strongly encouraged with Bu Css 900 ± 100 ng/mL/ or first AUC of 800-1400 μMol-min, but are not mandated by this protocol.

Cyclophosphamide (Cy) will be administered on Day -3 and Day -2 at a dose of 50 mg/kg per day intravenously (IV) if the patient is randomized to the PTCy arm, or at a dose of 60 mg/kg per day IV if the patient is randomized to the Control arm. Doses ≥ 5,000 mg must be infused IV over

2 hours. Lower doses may be administered over one hour.

Hydration prior to Cy may be given according to institutional standards. A **recommended** approach is as follows: Patients are instructed to increase fluids overnight before Cy administration. Hydration with normal saline at 3 ml/kg/hr IV will be started 2 hours prior to Cy, then the rate will be reduced to 2 ml/kg/hr for 1 hour pre-cyclophosphamide and continued at 2 ml/kg/hr for 8 hours post-Cy.

Mesna is required in patients on the post-transplant Cy arm. Mesna dose must be ≥ 80% of the total daily dose of Cy and given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy intravenously.

For patients in the Control Arm, use of Mesna and dosing will be done according to institutional standards. A recommended approach is as follows: Mesna dose of ≥ 80% of the total daily dose of Cy and given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy intravenously.

2.4.4. Busulfan and fludarabine (Bu/Flu)

Table 2.4.4. – Post-Transplant Cy and Control Arms: Busulfan and Fludarabine Regimen

Days -5 to -2	Busulfan: 4 mg/kg/day PO dose with Bu Css 900±100 ng/mL, total dose of 16mg/kg PO 3.2mg/kg/day IV total dose of 12.8mg/kg 130 mg/m^2/day 520 mg/m^2
Days -5 to -2	Fludarabine: 40 mg/m^2/day, total dose of 160 mg/m^2

The sequence of busulfan and fludarabine administration will be done according to institutional standards as long as the prescribed doses are the same as the allowable regimen above.

Fludarabine will be administered intravenously at a total dose of 160 mg/m^2 divided into four daily doses according to institutional practices.

2.4.5. Cyclophosphamide and total body irradiation (Cy/TBI)

2.4.5.1. The allowable Cy/ TBI regimen for the Post-Transplant Cy Arm is the following:

Table 2.4.5.1. – Post-Transplant Cy Arm: Cyclophosphamide and Total Body Irradiation Regimen

Days -7 to -4	TBI 1200–1420 cGy: Administered according to the schedules utilized by the participating clinical centers.
Days -3 to -2	Cyclophosphamide: 50 mg/kg/day, total dose of 100 mg/kg

2.4.5.2. The allowable Cy/ TBI regimen for the Control Arm is the following:

Table 2.4.5.2. – Control Arm: Cyclophosphamide and Total Body Irradiation Regimen

Days -7 to -4	TBI 1200–1420 cGy: Administered according to the schedules utilized by the participating clinical centers.
Days -3 to -2	Cyclophosphamide: 60 mg/kg/day, total dose of 120 mg/kg

The sequence of cyclophosphamide, TBI and TBI administration practices will be in accordance with institutional standards as long as the prescribed doses are the same as the allowable regimen above.

Fractionated TBI will be administered according to the schedules utilized by the participating clinical centers. Radiation sources, dose rates, details of lung shielding, and sites receiving boost radiation will also be defined by the institution. TBI may be delivered from either linear accelerator or Cobalt sources. Palifermin may be given to patients receiving TBI per institutional practices.

Mesna is required in patients on the post-transplant Cy arm. Mesna dose must be ≥ 80% of the total daily dose of Cy and given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after Cy intravenously.

For patients in the Control Arm, use of Mesna and dosing will be done according to institutional standards. A recommended approach is as follows: Mesna dose of ≥ 80% of the total daily dose of Cy and given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy intravenously.

2.4.6. Total body irradiation and etoposide (TBI/Etoposide) – **for the Control Arm only**

The allowable TBI/Etoposide regimen is the following:

Table 2.4.6. – Control Arm: Total Body Irradiation and Etoposide Regimen

Days -7 to -4	TBI 1200–1320 cGy: Administered according to the schedules utilized by the participating clinical centers.

Day -3	Etoposide 60 mg/kg: Administered at a single dose of 60 mg/kg

Fractionated TBI will be administered according to the schedules utilized by the participating clinical centers. Radiation sources, dose rates, details of lung shielding, and sites receiving boost radiation will also be defined by the institution. TBI may be delivered from either linear accelerator or Cobalt sources. Palifermin may be given to patients receiving TBI per institutional practices.

Etoposide will be administered at a single dose of 60 mg/kg on Day -3.

2.5. Hematopoietic Stem Cell Graft Collection

2.5.1. CD34 Selection Arm

Donors of patients randomized to the CD34 selection arm are considered research subjects according to the FDA regulations regarding human subjects (21 CFR Part 812.3). Unrelated donors will be consented after recipients are randomized. For unrelated donors, the National Marrow Donor Program (NMDP) will oversee the donor consent procedures by distributing the donor consent document to Donor Centers and maintaining IRB oversight of the consent procedures. For related donors, the consent process will be done at the transplant center along with routine donor consent procedures according to institutional guidelines. The donor informed consent document for related donors, including assent for minors, will be reviewed by each transplant center's IRB.

2.5.1.1. Mobilization of Donor

Following screening and enrollment, the donor of patients randomized to the CD34-selection arm will receive mobilization therapy with once daily G-CSF. Mobilization will begin on Day -5 of the conditioning regimen at a G-CSF dose of 10 µg/kg/day actual body weight subcutaneously (rounded off to a multiple of the nearest vial size of either 300 or 480 µg). Based on the volume, the dose may be split into 2 or 3 injection sites. The mobilization phase starts on the first day of administration of G-CSF and continues until the final day of leukapheresis.

2.5.1.2. Progenitor Cell Collection and Processing

Leukapheresis

Leukapheresis will be performed on a continuous flow cell separator according to institutional standards and will commence on the morning of the fifth day of G-CSF treatment. The anti-coagulant used for the procedure will be acid citrate dextrose (ACD). No additional anti-coagulants or additives (heparin, etc.) should be added beyond those normally used during leukapheresis. The volume of blood processed per leukapheresis session should be approximately three to four times total blood volume as tolerated by the donor. Concurrent plasma (200-300 mL) should be collected for products to be stored overnight after receipt into the processing facility. A unique identification and labeling system shall be used to track the leukapheresis product from collection to infusion according to FACT/JACIE guidelines.

For the purpose of cell infusion, the actual body weight will be used to calculate cell dose. A target dose ≥ 5.0 x 10^6 CD34+ cells/kg after selection containing ≤ 1.0 x 10^5 CD3+ cells/kg is desired. Up to two leukapheresis products containing a total of 10.0 x 10^6 CD34+ cells per kg may be needed to achieve this dose, assuming a 50% recovery of CD34+ cells after processing. A minimum of 4.0 x 10^6 CD34+ cells/kg must be collected to achieve an expected infusion dose of 2.0 x 10^6 CD34+ cells/kg.

For related donors - Decisions concerning the need for further product collection will be based on the known or projected enriched CD34+ cell content of the previously collected products. Data from the BMT CTN 0303 trial indicates a median recovery of CD34+ cells of 65% with more than 90% of selection procedures having a recovery ≥ 50%.[66] As a conservative estimate ≥10.0 x 10^6 CD34+ cells/kg recipient body weight at collection should result in the desired target infusion dose of 5.0 x 10^6 CD34+ cells/kg. The majority of patients will achieve this dose in a single apheresis collection and nearly all will achieve this dose with two collections. T-cell depletion was excellent in the BMT CTN 0303 study and it is not expected that T-cell content will exceed 1.0 x 10^5/kg with two collections. If after two collections the CD34+ cell dose is ≥ 2.0 x 10^6 per kg (the minimum required dose for infusion), no more collections will be performed. If the CD34+ cell dose is less than 4.0 X 10^6 cells/kg per kg CD34+ selection will not be performed.

A third collection to be infused without CD34-enrichment may be considered only if < 2.0 x 10^6 CD34+ cells/kg are expected to be available for infusion after two collections. If subsequent apheresis collection is not possible, or if minimum CD34 dose cannot be achieved without exceeding the maximum CD3 content, the patient will receive sufficient cells from the CD34-reduced fraction to reach the minimum CD34 dose.

If some or all of a third unmanipulated collection is infused or if cells from the CD34-reduced fraction are infused, the patient will be treated as if on the Control arm, receiving Tac/Mtx, but will be analyzed on the CD34+ selection arm, in accordance with the intent to treat principle.

For unrelated donors - The work up request to the donor center will include the CD34+ cell dose required of 10.0 x 10^6 CD34+ cells per kg, which falls within routine specifications from NMDP. The peripheral stem cell collection procedures will be according to NMDP procedures.

Transport and storage of product prior to processing

Analysis of processing data has indicated better results when processing is started within 36 hours of the end of collection. Therefore transport or storage times before the beginning of processing should target this upper limit. Products that will start processing within 24 hours of collection should be transported or stored at room temperature (19-25ºC). Products that require ≥24 hours of transport or storage should be maintained from the time of collection at refrigerator temperatures (1-8ºC) but must be warmed to room temperature prior to the start of processing.

CD34$^+$ selection with CliniMACS® device

Cell processing personnel will receive training by Miltenyi on the CliniMACS® System prior to activation of this protocol at their clinical site. The site will provide documentation to Miltenyi on

competency in the processing procedures, including the results of validation runs on the CliniMACS® System.

$CD34^+$ cell selection will be performed according to procedures given in the CliniMACS® Users Manual for the CliniMACS® CD34 Reagent System and the BMT CTN 1301 Standard Operating Procedures (SOPs) for CD34 Selection, in place and validated at the study sites. The CliniMACS® Users Manual can be found on the manufactures website (http://www.miltenyibiotec.com/en/clinical-applications/clinimacs-cd34-reagent-system-fda-approved/health-care-professionals/downloads-and-references.aspx).

Analysis of allograft

Samples will be taken from each leukapheresis product after collection and before processing and on each fraction of cells after processing on a single CliniMACS® tubing set. Pre- and post-$CD34^+$ selected cells shall be characterized as follows:

- Graft Evaluation (tests are performed as part of graft processing)
  - Gram stain (done locally) post-selection
  - Total nucleated cell count (done locally) pre- and post-processing
  - Endotoxin testing post-processing, done locally or sent to an authorized lab
  - Flow cytometric analysis for $CD34^+$ cells and for CD3+ cells pre- and post-processing done locally using validated SOPs
  - Flow cytometric analysis of monocytes, B cells, NK cells in the CD34-enriched products
  - Viability testing (7-AAD method or other fluorescent vital dye) pre- and post-processing
  - 14 day sterility cultures post-processing done locally using validated SOPs.
- Criteria for release of graft product for infusion. Note more than one CD34-enriched fraction may constitute a single graft product.
  - Viability ≥ 70% after selection
  - Negative gram stain
  - Cumulative $CD34^+$ cell content $\geq 2.0 \times 10^6$ per kg
  - Cumulative CD3+ cell content $\leq 1.0 \times 10^5$ per kg

As noted above, the target optimal allograft cell doses following processing on the CliniMACS® device include both a $CD34^+$ cell count of $> 5.0 \times 10^6$/kg recipient weight and a $CD3^+$ cell dose of $< 1.0 \times 10^5$/kg actual recipient weight. The targeted minimum $CD34^+$ cell dose following $CD34^+$ selection is 2.0×10^6/kg while the absolute minimum CD34+ cell dose is $\geq 1.0 \times 10^6$/kg.

It is also possible that doses of $CD34^+$ cells far exceeding 5×10^6/kg can be given without exceeding the maximum T-cell dose of 1.0×10^5 $CD3^+$ cells/kg. High doses of $CD34^+$ cells in

extensively T-cell depleted transplants are postulated to hasten immune reconstitution, without altering the low risk of GVHD. Consequently, there is no upper limit for the dose of $CD34^+$ cells/kg as long as the dose of $CD3^+$ cells does not exceed 1.0×10^5/kg.

Cryopreservation: If it is determined after randomization that cryopreservation may be needed, it must be approved by the protocol chairs.

2.5.1.3. Bone Marrow Collection and Processing

Bone marrow grafts will be used in patients who are randomized to either the PTCy or the Control arms. Bone marrow donors should undergo harvest in a time frame that will allow fastest delivery on Day 0. Either general or regional (epidural, spinal) anesthesia may be used. Donor bone marrow will be harvested with a target yield of 4×10^8 nucleated cells/kg recipient actual body weight. The CD 34^+ and T-cell count in the marrow will be quantified by flow cytometry according to institutional guidelines. Prior to the initiation of the transplant conditioning regimen, the estimated cell dose and a planned donor marrow volume shall be agreed upon by the donor and transplant center for unrelated donors; and between the transplant physician and cell processing laboratory for related donors.

Bone marrow processing, other than anticoagulation, filtration, packaging, and labeling in preparation for transportation, should not be performed by the collection center. The bone marrow graft will not be manipulated to deplete T cells. Patients will receive unprocessed marrow unless there is a major ABO incompatibility, in which case red blood cells will be depleted from the donor marrow using institutional practices. Institutional practices will determine if there will be processing for minor ABO incompatibilities. Processing for reduction of volume, plasma or fat may be performed by the transplant center according to institutional guidelines.

Cryopreservation: If it is determined after randomization that cryopreservation may be needed, it must be approved by the protocol chairs.

2.6. GVHD Prophylaxis

For patients who weigh less than 125% of their ideal body weight (IBW), dosing should be based on actual body weight. For patients who weigh greater than or equal to 125% of their IBW, dosing should be based on the adjusted ideal body weight (AIBW). These dosing rules do not apply to cyclosporine or tacrolimus, which should be dosed based on actual body weight.

2.6.1. Post-Transplant Cyclophosphamide

Hydration prior to cyclophosphamide may be given according to institutional standards. A recommended approach is as follows: Patients are instructed to increase fluids overnight before cyclophosphamide administration. Hydration with normal saline at 3 ml/kg/hr IV will be started 2 hours prior to cyclophosphamide, then the rate will be reduced to 2 ml/kg/hr for 1 hour pre-cyclophosphamide and continued at 2 ml/kg/hr for 8 hours post-cyclophosphamide.

Mesna is required in patients on the post-transplant Cy arm. Mesna dose must be ≥ 80% of the total daily dose of Cy and given in divided doses 30 minutes before and at 3, 6, and 8-9 hours after completion of Cy intravenously.

Cyclophosphamide [50 mg/kg] will be given on Day 3 post-transplant (**between 60 and 72 hours after marrow infusion**) and on Day 4 post-transplant (approximately 24 hours after Day 3 cyclophosphamide). Cyclophosphamide will be given as an IV infusion over 1-2 hours (depending on volume).

Systemic corticosteroids may not be used as an anti-emetic agent and should not be administered until 24 hours after the completion of post-transplantation cyclophosphamide, unless clinically indicated.

If patient develops GVHD on one of the CNI-free arms (PTCy or CD34 selection), the following treatment approach is recommended:

a. **Skin disease only** (< 50%BSA) – the recommended treatment is topical steroids.

b. **Skin disease only** (>50%BSA) – the recommended treatment is topical steroids with low threshold to escalate to systemic steroids. For systemic steroids, prednisone (1 to 2 mg/kg/day or IV equivalent) can be used. If no response after 72 hours or need for prolonged systemic steroids, the recommendation is to add tacrolimus (level 5-10 ng/mL).

c. **Grade 2 GI (upper or lower)** - the recommended treatment is a trial of budesonide (9 mg total daily). If no response within 7 days or progression within 72h, prednisone (2 mg/kg/day or IV equivalent) can be used, in addition to beclomethasone and/or budesonide for GI GVHD, and tacrolimus (level 5-10 ng/ml).

d. **Grade 3-4 GI (upper or lower) or hepatic GVHD:** the recommended treatment is prednisone (2 mg/kg/day or IV equivalent), in addition to beclomethasone and/or budesonide for GI GVHD, and tacrolimus (level 5-10 ng/ml).

2.6.2. CNI/Methotrexate Control Arm

2.6.2.1. Tacrolimus

Tacrolimus will be given orally or intravenously per institutional standards starting Day –3. The dose of tacrolimus may be rounded to the nearest 0.5 mg for oral formulations. Subsequent dosing will be based on blood levels, with a target of 5-15 ng/ml. If patients are on medications which alter the metabolism of tacrolimus (e.g. azoles), the initial starting dose and subsequent doses should be altered as per institutional practices. Tacrolimus taper can be initiated at a minimum of 90 days post HSCT if there is no evidence of active GVHD. The rate of tapering will be done according institutional practices but patients should be off tacrolimus by Day 180 post HSCT if there is no evidence of active GVHD.

Cyclosporine may be substituted for tacrolimus if the patient is intolerant of tacrolimus or per institutional practice. Cyclosporine will be administered IV beginning on Day -3 and doses will be adjusted to maintain a trough level of 200-400 ng/mL by HPLC or 250-500 ng/mL by TDX method (or 100-250 ng/mL by Tandem MS or equivalent level for other CSA testing methods) or within therapeutic level per institutional standard testing. If patients are on medications which alter the metabolism of cyclosporine (e.g. azoles), the initial starting dose and subsequent doses should be altered as per institutional practices. Cyclosporine taper can be initiated at a minimum of 90 days post HSCT if there is no evidence of active GVHD. The rate of tapering will be done according institutional practices but patients should be off cyclosporine by Day 180 post HSCT if there is no evidence of active GVHD.

2.6.2.2. Methotrexate

Methotrexate will be administered at the doses of 15 mg/m^2 IV bolus on Day +1, and 10 mg/m^2 IV bolus on Days +3, +6 and +11 after hematopoietic stem cell infusion. The Day +1 dose of methotrexate should be given at least 24 hours after the hematopoietic stem cell infusion ends. Dose reduction of MTX due to worsening creatinine clearance after initiation of conditioning regimen, high serum levels or development of oral mucositis is allowed according to institutional practices. Leucovorin rescue is allowed according to institutional practices.

2.7. Transplant Procedures

Unmanipulated BM or mobilized CD34-selected PBSC grafts will be administered on Day 0 to all patients according to individual institutional guidelines after appropriate processing and quantification has been performed by the local laboratory. Stem cells are administered through an indwelling central venous catheter. If infusion occurs over two days, Day 0 is the first day the infusion is initiated.

2.8. Supportive Care

All supportive care will be given in keeping with the BMT CTN Manual of Procedures and local institutional practice. Supportive care should be administered in a similar fashion to subjects randomized to all three arms of the study.

2.8.1. Growth Factors

G-CSF may be given per institutional guidelines.

2.8.2. Seizure Prophylaxis

Keppra (Levetiracetam) will be administered for the prevention of busulfan-associated seizures to all research participants receiving busulfan, starting Day –10 or 12 hours prior to starting busulfan. Dosing of Levetiracetam will be administered as per the BMT guidelines. Alternatively, ***Phenytoin*** **or another similar seizure prophylaxis agents** may be administered starting day –10 or 12 hours

prior to starting busulfan for the prevention of busulfan-associated seizures, according to institutional practices.

2.8.3. Blood Products

Transfusion thresholds for blood product support will be consistent with the BMT CTN MOP and standard institutional guidelines. All blood products will be irradiated.

2.8.4. Prophylaxis Against Infections

Patients will receive infection prophylaxis according to institutional guidelines. Infection prophylaxis should include, but is not limited to, agents or strategies (e.g., PCR screening and preemptive therapy) to reduce the risk of bacterial, herpes simplex, CMV, EBV, Pneumocystis jiroveci, toxoplasmosis, and fungal infections:

- Antifungal therapy: Prophylaxis with fluconazole or other antifungal agents can be given as per local institutional guidelines. **Fluconazole, voriconazole and other azoles are expected to increase serum cyclosporine or tacrolimus levels, therefore, dosages of cyclosporine or tacrolimus should be adjusted accordingly.**
- Cytomegalovirus (CMV**):** For patients on the CD34-selection arm, CMV monitoring through nucleic acid amplified testing (NAAT) will be done weekly through Day 100 and then at each clinical assessment until Day 180 post-transplant. For patients on the other 2 arms, CMV monitoring will be done according to institutional guidelines. It is *recommended* that weekly assessment for CMV be done through Day 63 post-transplant, and then at each clinical assessment until day 180 post-transplant. Any reactivation and/or CMV disease will be captured in this study. Preemptive treatment (early treatment of CMV viremia detected by PCR) is the preferred strategy for the majority of patients. It is recommended that the threshold to initiate preemptive therapy will be according to a value determined by the assay being performed at the institution or a rising trend on successive measurements from patient's baseline. As an example, when using an FDA-approved assay that has been calibrated, using WHO CMV standards, a threshold of 500 international units (IU)/mL may be used.
- Epstein-Barr Virus (EBV): For patients on the CD34-selection arm, EBV monitoring through nucleic acid amplified testing (NAAT) will be done weekly through Day 100 and then at each clinical assessment until Day 180 post-transplant. For patients on the other 2 arms, EBV monitoring will be done according to institutional guidelines. See below for management of patients with EBV viremia or lymphoproliferative syndrome (section 2.9.7.).
- Pneumocystis jiroveci: Prophylaxis with agents against Pneumocystis jiroveci can be given as per local institutional guidelines. In recipients of CD34-selected grafts, it is recommended that prophylaxis continue beyond day 180, until immune recovery, defined by a normal CD4 T cell count.

- Herpes virus (HSV or VZV): Patients must receive acyclovir or valacyclovir through Day 365 post-transplant as standard prophylaxis against HSV and VZV per institutional guidelines or until the CD4 T-cell count has normalized.
- Toxoplasmosis: For patients on the CD34-selection arm that are at risk (seropositive patient or donor), it is required that toxoplasmosis prophylaxis, through site preferred methods, begin on Day 30 post-transplant. Prophylaxis with agents against toxoplasmosis can be given as per local institutional guidelines. In recipients of CD34-selected grafts, prophylaxis should be given to patients at risk and continued beyond day 180, until immune recovery, defined by a normal CD4 T cell count.

2.8.5. Intravenous Immune Globulin (IVIG)

IVIG administration will be according to local institutional standard practice.

2.8.6. Sinusoidal Obstruction Syndrome (SOS)/Veno-occlusive Disease (VOD) of the Liver Prophylaxis

Prophylaxis against SOS/VOD with heparin and/or ursodiol will be according to local institutional standard practice.

2.8.7. Donor Lymphocyte Infusions – Viral-specific Cytotoxic T-Lymphocytes (CTLs)

Donor lymphocyte infusions (DLI) should only be performed for therapeutic reasons, including but not limited to relapsed or persistent disease or refractory infections. DLI should not be administered for mixed chimerism only.

Viral specific CTL (donor-derived or third-party) may be given for treatment of infections not responding to standard therapy (e.g. CMV, EBV, adenovirus, etc). For any investigational agent, the investigator will submit the research protocol to the BMT CTN 1301 protocol coordinator for review and approval by the protocol chairs/officer in order to co-enroll the patient on the non-BMT CTN study.

2.9. Participant Risks

2.9.1. Potential Sensitization to Murine Proteins

Mouse protein antibodies are used in the CliniMACS® processing procedures in the CD34 – selection arm. If the recipient has a pre-existing allergy, he or she may be at risk for allergic reactions during infusion of the processed cells, although the residual amount of murine protein in the final product is very low (estimated maximum dose for a 50 kg patient would be 30 µg). To date, no allergic reactions are reported in patients receiving cells processed with the CliniMACS® System. Epinephrine and antihistamines will be available at the recipient's bedside during the PBSC infusion.

2.9.2. Graft Infusion

Symptoms may include changes in heart rate and/or rhythm, changes in blood pressure, fever, chills, sweats, nausea, vomiting, diarrhea, abdominal cramping, hemoglobinuria, acute renal failure, allergic reactions, respiratory dysfunction, or headache.

2.9.3. Infections

Transplantation puts the patient at higher risk for bacterial, viral, or fungal infections, which are potentially life-threatening. These risks are potentially higher with CD-34 selected transplants. Prophylaxis will be initiated and patients will be closely monitored for signs of infections and will receive early and appropriate treatment**.**

2.9.4. Graft-Versus-Host Disease

Acute or chronic GVHD may develop after allogeneic transplantation that can be disabling and can lead to death. GVHD is thought to be initiated by T-cells contained in the graft. $CD34^+$ selection and PTCy reduce the number of alloreactive T-cells but GVHD can occur after these transplants.

2.9.5. Sinusoidal Obstruction Syndrome (SOS)/Veno-occlusive Disease (VOD) of the Liver

SOS/VOD is a manifestation of damage to the liver by the conditioning regimen that usually develops within two weeks after allogeneic transplant and is characterized by at least two of the following:

- Hyperbilirubinemia (total bilirubin > 2 mg/dL)
- Hepatomegaly or right upper quadrant pain, or
- Sudden weight gain (> 5% above baseline)

Recipients developing SOS/VOD will be monitored closely and will receive appropriate supportive care and careful fluid management. TCD is not expected to affect the risk of SOS/VOD.

2.9.6. End Organ Damage

End organ damage of all or any of the major organs, including the brain, may occur as a result of cumulative toxicity from anti-neoplastic therapy, reactions to other drugs, and as a result of destructive processes (e.g., infection, GVHD, etc.) and may have a fatal outcome. Toxicities may occur in any individual patient due to multiple events and cumulative effects that may involve any and all organs, including the brain. Brain damage can result in severe loss of cognitive or neurologic function. Data from previous studies do not suggest that the risk of end organ damage is appreciably affected by TCD or the preparative regimens to be used in this study.

2.9.7. Lymphoproliferative Syndrome

Recipients of CD34-selected allogeneic grafts have an increased risk of developing post-transplant lymphoproliferative disorder (PTLD) caused by EBV. Patients who develop a fever of unknown origin to > 39ºC, lymphadenopathy, or hepatosplenomegaly, should undergo CT scanning of the chest and abdomen and/or PET scan to rule out or stage EBV PTLD. Tissue diagnosis that includes EBER and LMP-1 immunocytochemistry should be attempted. Other diagnostic or staging studies will be performed as clinically indicated. EBV PTLD may rapidly progress and can be fatal if not treated. Management of suspected EBV PTLD should be discussed with one of the Protocol Chairpersons. EBV PTLD can be treated with rituximab and/or infusion of 10^6 T-cells/kg from the donor. It is recommended that patients with EBV DNA levels of > 1000 copies/mL receive 375 mg/m^2 of rituximab. Those patients that continue to have levels above 1000 copies/mL on subsequent testing should be considered to receive three additional weekly infusions of 375 mg/m^2 of rituximab. Patients with rapidly rising EBV DNA levels or clinical symptoms are recommended to have imaging studies to diagnose an EBV PTLD. An accelerated schedule of days 1, 4, 8, 15 can be used if there is a suspicion of EBV PTLD. Rituximab has been shown to induce regression in 50 - 70% of cases. Note: Rituximab does not enter the CNS and is not effective in treating CNS disease. Donor lymphocyte infusions may induce regression in > 90% of cases of EBV PTLD and are effective in CNS disease.

2.9.8. Death

There is an approximate 5-10% risk of transplant-related mortality within the first month of transplant due to the risk of severe regimen related toxicity, hemorrhage, opportunistic infection, or other complications. It is not expected that the regimens to be used in this protocol will increase this risk.

2.9.9. Therapy Toxicities

All toxicities will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. Below is a list of the most common toxicities for therapies used in this study. See the FDA-approved package insert for each drug for a comprehensive list of adverse events.

2.9.10. Busulfan

Busulfan (1, 4-dimethanesulfonoxybutane) is an alkylating agent. The drug is extensively metabolized and its metabolites are eventually excreted in the urine. The oral preparation is well absorbed but studies at this institution have indicated that there is a ten-fold variability area under the curve (AUC) of the drug among patients receiving busulfan by mouth. There is a statistical association between increased AUC and the development of veno occlusive disease of the liver. Since its FDA approval in 1999, IV Bu has been used increasingly in combination with CY or Flu.[17, 18, 19, 20] IV Bu was initially administered every 6-hours, similar to oral Bu. However, several studies have used the drug with once or twice daily administration. In terms of safety, IV Bu and oral Bu appear to have similar toxicity profiles. It has been proposed that sinusoidal obstruction syndrome and mucositis may be reduced in incidence and severity with IV Bu.[21] The IV formulation at a dose of 0.8 mg/kg IV every 6-hrs is considered equivalent to the oral formulation

at a dose of 1 mg/kg PO every 6 hrs in conditioning regimens. On this basis a regimen using 4x0.8 = 3.2 mg/kg as a single daily dose has been developed.[19, 22]

Toxicities associated with busulfan administration include:

- Gastrointestinal: nausea, vomiting, constipation, diarrhea, abdominal discomfort, anorexia, dyspepsia and mucositis
- Hepatobiliary: veno-occlusive disease
- Neurologic: headache, insomnia and seizures
- Cardiovascular: hypertension, hypotension and tachycardia
- Pulmonary: dyspnea, lung fibrosis
- Endocrine and metabolic: hypermagnesemia, hyperglycemia and hyperphosphatemia
- Miscellaneous: rhinorrhea, amenorrhea, infertility, skin rashes, cataracts

2.9.11. Cyclophosphamide

Cyclophosphamide (CY) is an alkylating agent which prevents cell division primarily by cross-linking DNA strands. CY is converted to its active form in vivo by hepatic enzymes. After a single dose, tissue enzymes degrade most of the active metabolites. After high doses (> 40 mg/kg), the alkylating activity in the plasma is minimal by 24 hours. Several of the metabolites appear to have toxic actions. One of the metabolic products, acrolein (CH_2=CH-CHO), is known to be toxic to the bladder urothelium and can cause hemorrhagic cystitis when CY is administered at high doses.

Some of the most common toxicities associated with cyclophosphamide include:

- Gastrointestinal: nausea, vomiting and anorexia
- Hematologic: myelosuppression
- Cardiovascular: severe chronic heart failure characterized by cardiomegaly, pericardial effusions, diffuse voltage decrease on ECG and decreased LVEF
- Genitourinary: hemorrhagic cystitis (prevented by hydration and mesna therapy or bladder irrigation) and gonadal function impairment
- Miscellaneous: fluid retention, alopecia and rare pulmonary toxicity

2.9.12. Etoposide

The most common side effects of etoposide are:

- Hematologic: myelosuppression
- Gastrointestinal: nausea, vomiting, anorexia, diarrhea, abdominal pain, stomatitis
- Dermatologic: alopecia
- Cardiovascular: hypotension
- Neurologic: peripheral neuropathy
- Miscellaneous: elevated liver function tests, allergic reactions, and second cancers including myelodysplasia

2.9.13. Fludarabine

Fludarabine is a fluorinated nucleoside analog. After phosphorylation to fluoro-ara-ATP the drug appears to incorporate into DNA and inhibit DNA polymerase alpha, ribonucleotide reductase and DNA primase, thus inhibiting DNA synthesis. Excretion of fludarabine is impaired in patients with impaired renal function.

Common side effects of fludarabine include:

- Hematologic: hematopoietic suppression including neutropenia with increased risk of infection and immunosuppression
- Neurologic: peripheral neuropathy and encephalopathy manifested by fatigue, weakness, paresthesia, visual disturbances, somnolence and coma
- Gastrointestinal: nausea, vomiting, diarrhea and stomatitis
- Miscellaneous: fever, skin rash, cough and idiopathic pneumonitis

2.9.14. Rabbit Anti-Thymocyte Globulin

The ATG to be used in this trial is a purified preparation of rabbit gamma globulin containing high concentrations of antibodies against human lymphocytes. The preparation may contain low levels of antibody that cross-react with human platelets, white cells or red cells. The potential side effects of ATG are:

- Hematologic: neutropenia and thrombocytopenia
- Dermatologic: skin rash and itching
- Neurologic: fever, chills,
- Miscellaneous: serum sickness (severe skin rashes, mouth and vaginal sores, pain and swelling of the joints, or kidney damage) and anaphylaxis (hypotension, wheezing, difficulty breathing and severe hives)

2.9.15. Melphalan

Common toxicities of melphalan include:

- Hematologic: bone marrow suppression and hemolytic anemia
- Gastrointestinal: severe stomatitis, esophagitis and diarrhea
- Pulmonary: pulmonary fibrosis and interstitial pneumonitis
- Dermatologic: skin hypersensitivity and alopecia
- Miscellaneous: vasculitis and allergic reactions

2.9.16. Total Body Irradiation

Common toxicities of total body irradiation include:

- Gastrointestinal: nausea, vomiting and diarrhea
- Hematologic: marrow suppression
- Dermatologic: reversible skin pigmentation and alopecia

- Late effects: cataract formation, growth retardation, pulmonary damage, carcinogenesis and sterilization
- Miscellaneous: fever and parotiditis

2.9.17. Thiotepa

The most common toxicities seen with thiotepa include:

- Gastrointestinal: nausea and vomiting
- Hematologic: myelosuppression
- Dermatologic: transient generalized skin erythema, mainly in the axillary and inguinal folds
- Neurologic: mild cognitive dysfunction, disorientation, confusion and irritability

2.9.18. Tacrolimus

Tacrolimus often called by its original drug code name, FK-506, is a macrolide immunosuppressant that inhibits calcineurin (phosphatase 2B)-mediated T-cell activation by forming a complex with FK506 binding protein 12 (FKBP12). Tacrolimus side effects include:

- Cardiovascular: hypertension
- Neurologic: confusion, dizziness, insomnia, seizures, tremors, changes in how clearly one can think
- Gastrointestinal: nausea, vomiting
- Hematologic: microangiopathic hemolytic anemia, thrombocytopenia
- Endocrine and metabolic: hypomagnesemia, hypokalemia, hypocalcemia, hyperlipidemia
- Miscellaneous: unwanted hair growth, changes in vision, liver problems, reversible renal insufficiency, infections and post-transplant lymphoproliferative disorders

2.9.19. Methotrexate

The most frequently reported adverse reactions associated with methotrexate use as GVHD prophylaxis include:

- Neurologic: fever, dizziness, chills, undue fatigue
- Gastrointestinal: ulcerative stomatitis, nausea, abdominal distress, diarrhea
- Hematologic: leucopenia, anemia, and suppressed hematopoiesis (leading to infection)
- Miscellaneous: abnormal liver tests, kidney failure, and pulmonary complications after transplantation

2.9.20. Cyclosporine

The most common side effects of cyclosporine are:

- Cardiovascular: hypertension
- Neurologic: paresthesias, neuropathy and seizures

- Hematologic: thrombotic microangiopathy
- Endocrine and metabolic: electrolyte imbalances
- Miscellaneous: hirsutism; hepatic and renal dysfunction; nephrotoxicity; gingival hyperplasia; transient blindness

2.9.21. MESNA (Sodium -2-Mercaptoethane Sulphonate)

Mesna is a prophylactic agent used to prevent hemorrhagic cystitis induced by the oxazophosphorines (cyclophosphamide and ifosfamide). It has no intrinsic cytotoxicity and no antagonistic effects on chemotherapy. Mesna binds with acrolein, the urotoxic metabolite produced by the oxazophosphorines, to produce a non-toxic thioether and slows the rate of acrolein formation by combining with 4-hydroxy metabolites of oxazophosphorines. At the doses used for uroprotection, mesna is virtually non-toxic. The most common side effects of MESNA are:

- Cardiovascular: hypotension
- Dermatologic: rash, urticarial
- Gastrointestinal: nausea and vomiting, diarrhea, abdominal pain, altered taste
- Neurologic: headache, joint or limb pain
- Miscellaneous: fatigue

2.10. Quality of Life Assessments

2.10.1. Instruments

FACT-BMT: The Functional Assessment of Cancer Therapy – Bone Marrow Transplant subscale[67] version 4.0 instrument is a 37 item scale comprised of a general core questionnaire, the FACT-G, that evaluates the health-related quality of life (HQL) of patients receiving treatment for cancer, and a specific module, BMT Concerns, that addresses disease and treatment-related questions specific to bone marrow transplant. The FACT-G consists of four subscales developed and normed in cancer patients: Physical Well-being, Social/Family Well-being, Emotional Well-being, and Functional Well-being. Each subscale is positively scored, with higher scores indicating better functioning. The FACT-BMT Trial Outcome Index, comprised of the physical well being scale, the functional well being scale and the BMT specific items, will be used as the outcome measure in summarizing the FACT-BMT data. The FACT-BMT takes 6 minutes to complete, and is routinely used in BMT CTN trials.

MOS SF-36: The Medical Outcomes Study Short Form 36 is a 36 item general assessment of health quality of life with eight components: Physical Functioning, Role Physical, Pain Index, General Health Perceptions, Vitality, Social Functioning, Role Emotional, and Mental Health Index. Each domain is positively scored, indicating that higher scores are associated with positive outcome. This scale has been widely applied in a variety of outcome studies and is being used in this protocol as a generic measure of quality of life. To facilitate comparison of the results with published norms, the Physical Component Summary (PCS) and Mental Component Summary (MCS) will be used as the outcome measures in summarizing the SF-36 data. These summary scores are derived by multiplying the z-score for each scale by its respective physical or mental

factor score coefficient and summing the products. Resulting scores are then transformed into T-scores (mean=50; standard deviation=10). The SF-36 takes 6 minutes to complete.[68, 69]

MDASI: The MD Anderson Symptom Inventory is a 19 item instrument that captures 13 symptoms (0="not present" to 10="as bad as you can imagine") and 6 items measuring interference with life from 0 ("did not interfere") to 10 ("interfered completely"). It provides two summary scales: symptoms and interference.[70] The MDASI takes less than 5 minutes to complete, and was collected in the BMT CTN 0802 trial of acute GVHD treatment.

PedsQL: The PedsQL™ Stem Cell Transplant Module is a 46 item instrument that measures health-related quality of life in children and adolescents undergoing hematopoietic stem cell transplant, and is developmentally appropriate for self-report in ages 8 through 18 years.

2.10.2. Administration

The self report questionnaires will be completed at Baseline, then at Day 100, Day 180, 12 months and 24 months post-transplant or until death. Only English speaking adult and pediatric patients, and Spanish speaking adult patients are eligible to participate in the Health Quality of Life (HQL) component of this trial. Patients >18 years will complete the FACT-BMT, MOS SF-36 and MDASI instruments. Patients ≥ 8 years through 18 years will complete the PedsQL™ Stem Cell Transplant Module. Surveys are completed by participants using self-completed instruments as a first choice. If this method of data collection is not possible, then surveys and response options may be read verbatim to participants, either in person or over the phone, to collect data. The method of survey completion, the date, and the language will be recorded in the database. **Surveys may not be completed by surrogates**.

TABLE 2.10 – REQUIRED PATIENT-REPORTED OUTCOMES DATA COLLECTION[1]

Instrument	Number of items	Baseline	Day 100	Day 180	12 months	24 months
FACT-BMT	37	X	X	X	X	X
MOS SF-36	36	X	X	X	X	X
MDASI	19	X	X	X	X	X
PedsQL™ SCT Module	46	X	X	X	X	X

[1]Surveys will be completed only by English speaking adult and pediatric patients, and Spanish speaking adult patients

CHAPTER 3

3. STUDY ENDPOINTS

3.1. Primary Endpoint

The primary endpoint is chronic GVHD/relapse-free survival (CRFS). All randomized patients will be analyzed for this endpoint from the date of randomization. An event for this time to event outcome is defined as moderate to severe chronic GVHD according to NIH consensus criteria global score, disease relapse, or death by any cause. Patients will be followed up for two years for this endpoint.

3.2. Secondary Endpoints

3.2.1. Overall Survival

Overall survival is defined as the time interval between date of transplant and death from any cause or for surviving patients, to last follow-up. The event for this endpoint is death from any cause.

3.2.2. Disease Relapse

Relapse is defined by either morphological evidence of acute leukemia or MDS consistent with pre-transplant features, documented or not by biopsy. The event is defined as increase in size of prior sites of disease or evidence of new sites of disease, documented or not by biopsy.

Acute leukemia and MDS – Relapse will be diagnosed when there is morphologic or clinical evidence of disease:

- Reappearance of leukemia blast cells in the peripheral blood; or,
- >5% blasts in the bone marrow, not attributable to another cause (e.g. bone marrow regeneration); or
- The appearance of previous or new dysplastic changes (MDS specific) within the bone marrow with or without falling donor chimerism; or
- The development of extramedullary leukemia or leukemic cells in the cerebral spinal fluid; or

Institution of any therapy to treat persistent or relapsed disease, including donor lymphocyte infusion, will be considered evidence of relapse regardless of whether the criteria described above were met. Two exceptions are the administration of FLT3 and other tyrosine kinase inhibitors for post-transplant maintenance and for prevention of disease relapse in patient with or without minimal residual disease identified after transplant.

3.2.3. Relapse-Free Survival

Relapse-free survival is the time from date of transplant to death or relapse, whichever comes first. The event for this endpoint is relapse or death. Patients alive and free from disease relapse will be censored at last follow-up.

3.2.4. Transplant-related Mortality

The cumulative incidence of transplant-related mortality (TRM) will be estimated at Days 100, 180, and 1 year after HSCT. An event for this endpoint is death without evidence of disease recurrence. Disease recurrence will be considered a competing event.

3.2.5. Immunosuppression-free Survival

Patients who are alive, relapse-free, and do not need ongoing immune suppression to control GVHD at one year post HSCT are considered successes for this endpoint. Immune suppression is defined as any systemic agents used to control or suppress GVHD. Corticosteroid doses of prednisone >10 mg, or equivalent (0.15 mg/kg for pediatrics) will be considered active systemic immune suppression treatment. Patients who discontinued immune suppression ≤ 15 days prior to the 1-year time point will be considered to be on immune suppression for this endpoint. Additionally the burden of immunosuppression in one year defined as the number of agents, the line of therapy, dose and duration of therapy will be described for all patients.

3.2.6. Hematologic Recovery

Hematologic recovery will be assessed according to neutrophil and platelet counts recovery after HSCT. Neutrophil recovery is defined as achieving an absolute neutrophil count (ANC) ≥ 500/mm^3 for three consecutive measurements on three different days. The first of the three days will be designated the day of neutrophil recovery. The competing event is death without neutrophil recovery. For patients who never drop ANC below 500/mm^3, the date of neutrophil recovery will be Day +1 post HSCT.

Platelet recovery is defined as the first day of a sustained platelet count >20,000/mm^3 with no platelet transfusion in the preceding seven days. The first day of sustained platelet count above this threshold will be designated the day of platelet engraftment. For patients who never drop their platelet count below 20,000/mm^3, the date of platelet recovery will be Day +1 post HSCT.

3.2.7. Graft Failure

Graft failure will be assessed as secondary endpoints, including primary and secondary graft failure. Primary graft failure is defined as no neutrophil recovery to ≥ 500 cells/µL by Day 28 post HSCT. Secondary graft failure will be assessed according to neutrophil count after initial hematologic recovery. Secondary graft failure is defined as initial neutrophil engraftment followed by subsequent decline in absolute neutrophil counts < 500 cells/µL, unresponsive to growth factor therapy, but cannot be explained by disease relapse or medications. Assessment for this endpoint will occur up to two years post HSCT.

3.2.8. Acute GVHD

Cumulative incidences of grade II-IV and III-IV acute GVHD will be determined. Acute GVHD will be graded according to the BMT CTN MOP. The time of onset of acute grades II-IV and III-IV acute GVHD will be recorded, as well as the maximum grade achieved. This endpoint will be evaluated through 180 days post HSCT.

3.2.9. Chronic GVHD

The cumulative incidence of chronic GVHD will be determined. Data will be collected directly from providers and chart review according to the recommendations of the NIH Consensus Criteria. Eight organs will be scored on a 0-3 scale to reflect degree of chronic GVHD involvement. Liver and pulmonary function test results and use of systemic therapy for treatment of chronic GVHD will also be recorded. These data will allow calculation of the NIH global severity scores of mild, moderate and severe chronic GVHD. Assessment of development of chronic GVHD will occur up to two years post HSCT.

3.2.10. Chronic GVHD-Free Survival

The event for this endpoint includes moderate to severe chronic GVHD according to NIH consensus criteria global score, or death by any cause. Assessment for this endpoint will occur up to two years post HSCT.

3.2.11. Toxicity

All grades ≥ 3 toxicities according to CTCAE, version 4 will be tabulated for each intervention arm. The proportion of patients developing grade ≥ 3 AE across intervention arms will be compared. Additionally incidences of hepatic sinusoidal obstruction syndrome/veno-occlusive disease (SOS/VOD) or idiopathic pneumonia syndrome (IPS) will also be described. SOS/VOD defined as the presence of at least two of the findings listed below within the first 25 days from transplant:

- Hyperbilirubinemia (total bilirubin > 2 mg/dL)
- Hepatomegaly or right upper quadrant pain, or
- Sudden weight gain (> 5% above baseline)

Clinical suspicion of SOS/VOD after 25 days might require further radiologic evidence of compromised portal flow or biopsy to rule out other diagnosis.

IPS is defined as presence of pneumonia like clinical picture with rapid decline of pulmonary function without evidence of infectious etiology, usually occurring within the first 100 days post-transplant.

3.2.12. Infections

The incidence of definite and probable viral, fungal and bacterial infections will be tabulated for each intervention arm. All Grade 2 and 3 infections will be reported according to the BMT CTN MOP. Infections of interest, including reactivation of CMV with or without organ involvement will be capture and described. Reactivation is defined as the presence of >1000 copies/mL or 500 IU/mL of CMV in the blood by NAAT. In addition, in the CD34-selected arm, reactivation of EBV (defined as the presence of >1000 copies in the blood by NAAT) with or without organ involvement will also be captured and described. Organ involvement requires assessment of pathologic specimens. Furthermore, for CMV and EBV, a decision to institute treatment by the patient's physician will also be considered an infection.

3.2.13. Immune Reconstitution

It is proposed that quanitative assessments of peripheral blood CD3, CD4, CD8, CD19 and CD56 positive lymphocytes will be performed through flow cytometric analysis at a central lab on *optional* research samples collected at Days 35, 100, 180, and 365 after transplant. Qualitative assessments will be tabulated according to time from transplant (see Appendix C).

3.2.14. Health-Related Quality of Life

HQL will be measured at Baseline and then at Days 100, 180, 365, and 730 post-transplant using four instruments: the SF36, FACT-BMT, and MDASI for adult patients (> 18 years), and the PedsQL™ Stem Cell Transplant Module for pediatric patients (≥ 8 years through 18 years). The instruments will be scored according to the recommendations of the developers. See section 2.10.1 for detailed descriptions of the instruments. The FACT-BMT instrument will be summarized by the Trial Outcome Index, comprised of the physical, functional, and BMT-specific items. The SF36 will be summarized by the Physical Component Summary (PCS) and Mental Component Summary (MCS). HQL will be described and compared between the treatment arms over time. Only adult patients able to read and speak in English or Spanish, and pediatric patients able to read and speak in English, are eligible to participate in the HQL component of this trial.

CHAPTER 4

4. PATIENT ENROLLMENT AND EVALUATIONS

4.1. Approaching Patients, Eligibility Screening and Obtaining Consent

Subjects will be approached for this study after the decision to proceed with transplantation is made and a suitable HLA-matched donor is identified. Patients willing to participate in the trial will sign an Institutional Review Board approved consent form. For patients with unrelated donors, it is recommended a hold for workup request be submitted to the NMDP at this time. Transplant physicians will evaluate the patient eligibility for randomization onto this study (see Section 2.3). Eligibility criteria will be verified and ineligible patients will not be randomized and no follow-up will be obtained. At the time of enrollment, donors without known contraindications or preferences for a specific stem cell source will be selected to donate. Transplant center personnel will record the documentation of patient consent and eligibility criteria in Emmes AdvantageEDC^{SM} (Electronic Data Capture, an Internet-based data entry system). For patients with unrelated donors, the transplant center staff will submit an unrelated donor work up request for the specific stem cell graft and dose according to randomization results.

Donors of patients randomized to the CD34+ selection arm will be required to sign an additional donor consent prior to donation. The transplant center personnel will be responsible for consenting related donors to patients randomized to the CD34+ selection arm. For patients with unrelated donors randomized to this arm, a Request for NMDP Donor to Participate in a Research Study form must also be submitted to the NMDP. The NMDP will be responsible for overseeing the consent procedures among unrelated donors to patients randomized to the CD34+ selection arm.

4.2. Randomization

Once the patient is deemed eligible and has given written informed consent, and the transplant center has confirmed the donor match grade by entering the recipient and donor HLA typing in AdvantageEDC^{SM}, the patient will be randomized to a treatment arm (CD34 selection, post-transplant cyclophosphamide or control). Once the randomization has occurred, the transplant center will submit the unrelated donor workup request to the NMDP, or initiate their related donor workup process. **Initiation of conditioning regimens should occur as soon as possible after randomization.**

If there is an unexpected delay in initiation of conditioning, certain pre-transplant evaluations may have to be repeated. Refer to section 4.4.1.1 Patient Assessments-Pre-Transplant Evaluations.

After randomization, if the donor changes his or her mind or refuses to donate the requested graft, the patient will be treated according to the Control arm, with calcineurin inhibitor-based GVHD prophylaxis (Tax/Mtx). This patient will be followed for all study outcomes and will be analyzed according to the “intent to treat” principle in the arm to which the patient was randomized.

4.3. Treatment Scheduling

Treatment should be initiated as soon as possible after randomization. This will prevent subject attrition prior to HSCT for reasons such as disease progression.

4.4. Study Monitoring

The follow-up schedule for scheduled study visits is outlined in Table 4.4a. A detailed description of each of the forms and the procedures required for forms completion and submission can be found in the Data Management Handbook and User's Guide.

TABLE 4.4a: FOLLOW-UP SCHEDULE

Study Visit	Target Day Post-Transplant
Baseline	≤ 42 days from randomization
1 week	7 ± 3 days
2 week	14 ± 3 days
3 week	21 ± 3 days
4 week	28 ± 3 days
5 week	35 ± 3 days
6 week	42 ± 3 days
7 week	49 ± 3 days
8 week	56 ± 3 days
9 week	63 ± 3 days
100 day	100 ± 7 days
5 month	150 ± 7 days
6 month	180 ± 28 days
9 month	270 ± 28 days
12 month	365 ± 28 days
24 month	730 ± 28 days

4.4.1. Patient Assessments

Table 4.4b summarizes patient clinical assessments over the course of the study.

TABLE 4.4b: PATIENT CLINICAL ASSESSMENTS

This table is offered as a visual *overview* of expected assessments and their time points during the course of this study. Please refer to Sections 4.4.1.1. and 4.4.1.2. for details of each assessment.

Study Assessments/ Testing	Baseline	7	14	21	28	35	42	49	56	63	100	150	180	270	365	730
History, physical exam, weight and height[1]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Karnofsky or Lansky performance status	X										X		X	X	X	X
HCT-Specific Co-Morbidity Index (HCT-CI) score	X															
HLA typing (recipient and donor)	X															
CBC[2], differential, platelet count, and blood chemistries[3]	X	X	X	X	X	X	X	X	X	X	X		X	X	X	
Estimated creatinine clearance[4]	X															
Infectious disease markers [5]	X															
Cardiac assessments[6]	X															
Pulmonary function tests[7]	X												X		X	X
Disease evaluation[8]	X										X		X		X	X
Chest x-ray or chest CT	X															
Pregnancy test[9]	X															
GVHD assessments[10]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Toxicity assessments[11]					X				X		X		X	X	X	X
CMV Monitoring (Refer to Section 2.8.4.)		X	X	X	X	X	X	X	X	X	X	X	X			
EBV Monitoring (Refer to Section 2.8.4.)		X	X	X	X	X	X	X	X	X	X	X	X			
Quality of Life assessments[12]	X										X		X		X	X
Optional Research Samples[13]	X					X					X		X		X	

[1]Height is only required at the Baseline visit.

[2]CBC and manual WBC differential performed three times weekly from Day 0 until ANC > 500/mcL for three days and platelet count > 20,000/mcL after nadir, while hospitalized. CBC then performed weekly through Day 63 post-transplant and every other week through Day 100 post-transplant, then at Days 180, 270 and 365 post-transplant.

[3]Blood chemistries include: serum creatinine, bilirubin, alkaline phosphatase, AST and ALT. Blood chemistries performed twice weekly until hospital discharge. Blood chemistries performed weekly after hospital discharge until Day 63 post-transplant, then every other week through Day 100 post-transplant, and then at Days 180, 270 and 365 post-transplant.

[4]Estimated creatinine clearance for patients > 12 years is calculated using the Cockroft-Gault formula and actual body weight; the updated Schwartz formula should be used for pediatric patients (≥ 1 year to 12 years). See Appendix D for estimated creatinine clearance formulas).

[5]Infectious disease markers include: CMV, EBV, Hepatitis panel (HepA Ab, HepB SAb, HepB SAg, HepB Core Ab, HepC Ab), herpes simplex virus, syphilis, HIV-1 and -2 and HTLV-I and -II antibody, varicella zoster, and toxoplasmosis (IgG and IgM).

[6]Cardiac assessments include EKG and left ventricular ejection fraction (LVEF) or shortening fraction by echocardiogram or radionuclide scan (MUGA).

[7]Pulmonary function tests include DLCO (Adj for Hgb), FEV1 and FVC. For children who are unable to perform for PFTs due to age or developmental ability, there must be no evidence of dyspnea at rest and no need for supplemental oxygen (as evidenced by O_2 saturation > 92% on room air) at baseline in order to meet eligibility criteria.

[8]Disease evaluation for acute leukemia and MDS includes a bone marrow aspirate and biopsy. Cytogenetics, molecular, or other institutional assessment to determine Minimal Residual Disease (MRD) will be recorded at Baseline to document patient's pre-transplant MRD status.

[9]Pregnancy test must be performed ≤ 30 days before the start of the transplant conditioning regimen. Pregnancy test is required for females of child-bearing potential (i.e., not postmenopausal or surgically sterile), and may be performed per institutional practices.

[10]GVHD assessments performed weekly until Day 63 post-transplant, and then at Days 100, 150, 180, 270, 365, and 730. The GVHD assessment will include a review of **all** abnormalities experienced **during the entire assessment period** and the **highest grade** for each abnormality (*whether attributed to GVHD or not*) during the assessment period will be recorded on the Acute GVHD form and/or the Follow-up GVHD form in AdvantageEDC.

[11]The toxicity assessment will include a review of **all** toxicities experienced **during the entire assessment period** and the **highest grade** for each toxicity during the assessment period will be recorded on the Toxicity form in AdvantageEDC.

[12]QOL assessments include self-reported patient questionnaires: SF-36, FACT-BMT, and MDASI for English and Spanish speaking patients > 18 years, and PedsQL Stem Cell Transplant Module for English speaking pediatric patients (ages 8 through 18 years).

[13]Optional Research Sample collection for the BMT CTN Repository for patients who weigh > 30.0 kg and who consent to provide samples (see Appendix C).The Baseline sample will be collected prior to initiation of the conditioning regimen.

4.4.1.1. Pre-Transplant Evaluations

The following observations must be made ≤ 28 days prior to study randomization, and ≤ 56 days from the planned initiation of conditioning, unless otherwise indicated. If after randomization there is a delay in the initiation of conditioning, pre-transplant evaluations must be repeated according to institutional practice, unless otherwise indicated.

- History, physical examination, height and weight.
- Karnofsky or Lansky performance status and HCT-Specific Co-Morbidity Index (HCT-CI) score.
- CBC with WBC differential and platelet count, serum creatinine, bilirubin, alkaline phosphatase, AST and ALT.
- Estimated creatinine clearance, using the Cockroft-Gault formula and actual body weight for patients > 12 years; the Schwartz formula will be used for pediatric patients (≥ 1 year to 12 years).
- Infectious disease markers to include: CMV antibody, EBV antibody, Hepatitis panel (HepA Ab, HepB SAb, HepB SAg, HepB Core Ab, HepC Ab), herpes simplex virus, syphilis, HIV-1 and -2, and HTLV-I and -II antibody and varicella zoster. For patients randomized to the CD34 Cell Selection Arm, toxoplasmosis (IgG and IgM) must also be performed ≤ 56 days from the planned initiation of conditioning.
- CD34+ Selection arm only: Donor toxoplasmosis serologic evaluation (IgG and IgM). For both related and unrelated donors, toxoplasmosis testing will be performed at the transplant center. The transplant center must request additional 'day of collection' samples from the NMDP for unrelated donors in order to conduct the testing at their center. Donor testing must be performed on or before day of transplant.
- EKG and left ventricular ejection fraction (LVEF) or shortening fraction by echocardiogram or radionuclide scan (MUGA). Cardiac evaluations may be performed ≤ 56 days prior to randomization and ≤ 84 days prior to the planned initiation of conditioning.
- Pulmonary function tests, including DLCO (adjusted for hemoglobin), FEV1, and FVC. For children who are unable to perform for PFTs due to age or developmental ability, there must be no evidence of dyspnea at rest and no need for supplemental oxygen, as evidenced by O_2 saturation ≥ 92% on room air. Pulmonary function assessments may be performed ≤ 56 days prior to randomization and ≤ 84 days prior to the planned initiation of conditioning.
- Disease evaluation: bone marrow aspirate for pathology and cytogenetics. Cytogenetics, molecular, or other institutional assessment to determine Minimal Residual Disease (MRD) will be recorded at Baseline to document patient's pre-transplant MRD status. **Note: Bone marrow aspirate must be performed ≤ 30 days prior to randomization and must be repeated if not within 30 days prior to the initiation of the transplant conditioning regimen.** For MDS patients, Bone marrow aspirate must be performed ≤ 6 weeks prior to randomization and must be repeated if not within 6 weeks prior to the initiation of the transplant conditioning regimen.

- Chest X-ray or chest CT.
- Pregnancy test per institutional practices for females of child-bearing potential (i.e., not postmenopausal or surgically sterile). **NOTE: pregnancy test must be performed ≤ 30 days prior to randomization and must be repeated if not within 30 days prior to the initiation of the transplant conditioning regimen.**
- Quality of Life, patient 'self-reported' assessments to include the Functional Assessment of Cancer Therapy – Bone Marrow Transplant (FACT-BMT), the Medical Outcomes Study Short Form 36 (SF-36), and the MD Anderson Symptom Inventory (MDASI) for English and Spanish speaking adult patients (≥ 18 years). English speaking pediatric patients (ages 8 through 18 years) will complete the PedsQL™ Stem Cell Transplant Module. Quality of Life assessments may be completed after the patient has provided consent and at any time prior to the onset of conditioning.
- *Optional* peripheral blood samples for future research (limited to patients who weigh greater than 30.0 kg.) Samples must be obtained after the patient has provided consent and prior to the initiation of the conditioning regimen (Appendix C).

4.4.1.2. Post-Transplant Evaluations

The following observations should be made according to Table 4.4b:

- History and physical exam weekly through Day 63 post-transplant, then at Days 100, 150, 180, 270, 365 and 730 post-transplant.
- Toxicity assessments at Days 28, 56, 100, 180, 270, 365 and 730 post-transplant.
- GVHD assessments:
    - Clinical assessments weekly from Day 7 until Day 63 post-transplant, then at Days 100, 150, 180, 270, 365 and 730 post-transplant. GVHD should be monitored in accordance with BMT CTN guidelines as specified in the BMT CTN Manual of Procedures (BMT CTN MOP).
    - Pulmonary function tests at Days 180, 365 and 730 post-transplant.
- Data on occurrence of infections and recorded as per the BMT CTN MOP.
    - CMV Monitoring: For patients on the CD34-selection arm, CMV monitoring through nucleic acid amplified testing (NAAT) will be done weekly through Day 100 and then at each clinical assessment until Day 180 post-transplant. For patients on the other 2 arms, CMV monitoring will be done according to institutional guidelines. It is *recommended* that weekly assessment for CMV be done through Day 63 post-transplant, and then at each clinical assessment until day 180 post-transplant. However, if both the recipient and the donor are CMV negative prior to transplant, CMV monitoring is not recommended unless clinically indicated. See Section 2.8.4. for details.
    - EBV Monitoring: For patients on the CD34-selection arm, EBV monitoring through nucleic acid amplified testing (NAAT) will be done weekly through Day

100 and then at each clinical assessment until Day 180 post-transplant. For patients on the other 2 arms, EBV monitoring will be done according to institutional guidelines. See Section 2.8.4. for details.

- CBC and WBC differential performed at least three times a week from Day 0 until ANC > 500/µL for 3 days and platelet count > 20,000/µL for 3 days (while hospitalized only) after nadir is reached. Manual WBC differential is not required if WBC < 500/µL. Thereafter, CBC weekly until Day 63 post-transplant, then every other week through Day 100 post-transplant, and then at Days 180, 270 and 365 post-transplant.
- Serum creatinine, bilirubin, alkaline phosphatase, ALT and AST, twice a week until hospital discharge. Thereafter, weekly until Day 63 post-transplant, then every other week through Day 100 post-transplant, and then at Days 180, 270 and 365 post-transplant.
- Disease evaluation: bone marrow aspirate for pathology and cytogenetics at Days 100, 180, 365 and 730 post-transplant.
- Quality of Life, patient 'self-reported' assessments at Days 100, 180, 365 and 730 post-transplant to include the Functional Assessment of Cancer Therapy – Bone Marrow Transplant (FACT-BMT), the Medical Outcomes Study Short Form 36 (SF-36), and the MD Anderson Symptom Inventory (MDASI) for English and Spanish speaking adult patients (> 18 years). English speaking pediatric patients (ages 8 through 18 years) will complete the PedsQL™ Stem Cell Transplant Module.
- *Optional* peripheral blood samples for future research (limited to patients who weigh greater than 30.0 kg.) at Days 35, 100, 180 and 365 post-transplant (Appendix C).

4.4.2. Criteria for Forms Submission

4.4.2.1. Electronic Case Report Forms (eCRFs)

All data for patients are recorded in the electronic Case Report Forms (eCRF) exclusively designed for the study. The Principal Investigator at each of the participating centers are responsible for complete, accurate, and timely reporting of data.

Criteria for timeliness of submission for all study forms are detailed in the Data Management Handbook and User's Guide. Forms that are not entered into AdvantageEDC within the specified time will be considered delinquent. A missing form will continue to be requested either until the form is entered into the AdvantageEDC and integrated into the Data and Coordinating Center's (DCC) master database, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Data Management Handbook.

Corrections in the eCRF are to be conducted only by authorized personnel and may require authorization prior to implementation of corrections. However, all earlier entries are retrievable despite corrections. All corrections are recorded automatically concerning date, time point and person. Plausibility and completeness of the eCRF are verified by personnel at the Data Coordinating Center.

At all times, the Principal Investigators at the participating centers have full responsibility for ensuring accuracy and authenticity of all clinical and laboratory data entered on the eCRFs.

4.4.2.2. Reporting Patient Deaths

Recipient death information must be entered into AdvantageEDC within 24 hours of knowledge of the patient's death. If the cause of death is unknown at that time, it need not be recorded at that time. However, once the cause of death is determined, the form must be updated in AdvantageEDC.

4.4.2.3. CIBMTR Data Reporting

Centers participating in BMT CTN trials must register pre- and post-transplant outcomes on all consecutive hematopoietic stem transplants done at their institution during their time of participation to the Center for International Blood and Marrow Transplant Research (CIBMTR). Registration is done using procedures and forms of the Stem Cell Transplant Outcomes Database (SCTOD) (Note: Federal legislation requires submission of these forms for all US allotransplant recipients). Enrollment on BMT CTN 1301 must be indicated on the SCTOD pre-transplant registration form. Additionally, CIBMTR pre- and post-transplant Report Forms must also be submitted for all patients enrolled on this trial. CIBMTR forms will be submitted directly to the CIBMTR at the times specified on the Form Submission Schedule.

4.4.3. Access to Data

Participating sites and their Principal Investigators must agree to allow trial-related on-site monitoring, including audits and regulatory inspections by DCC personnel such that the DCC has direct access to source data/documents as required.

4.4.4. Record Retention

Responsibilities of the Sponsor: As required by law, all study documents must be stored by the sponsor for at least 10 years after the clinical trial was finished or stopped.

Responsibilities of the Principal Investigator: The Investigator agrees to keep records, including the identity of all participating patients, all original signed informed consent forms, copies of all CRFs, source documents, detailed records of treatment disposition and other trial-related documents. The trial-related records should be retained by the Investigator for at least 10 years or as specified by contract, whichever is longer. In order to comply with the German Transfusion Law requirements, all participating German centers must ensure that the information and documents relating to the traceability for all the stages from donation of the hematopoietic cells to infusion into the recipient (including blood products and the graft [bone marrow or PBSC]) are archived in safe custody for a minimum of 30 years, even if the patient withdraws consent.

4.4.5. Study Monitoring

Monitoring and audits will be performed during the clinical study to ensure that the study meets the quality criteria.

The investigator agrees that the monitor will visit the study center in appropriate intervals. During these visits, the monitor will check the quality of the data recording and ensure that the study center adheres to the timeframe as set in the study protocol. The investigators agree to provide any relevant information and documentation whenever requested by the monitor. This includes access to all original study documents and source data including access to electronic source documents if necessary. Source data are checked and compared with entries in the data base. The participant has given consent to this procedure by signing the patient information and written informed consent form.

The monitor has the responsibility to treat all information confidentially and to safeguard the integrity and personal privacy of the study participants.

Following a monitoring visit, the Monitor will provide a report to the sponsor and the site, which will summarize the documents reviewed and a statement of findings, deviations, deficiencies, conclusions, actions taken and actions required. The Principal Investigator at each site will be responsible for ensuring that monitoring findings are addressed (this may be delegated to an appropriate member of staff).

Details of monitoring activities will be included in the Site Monitoring Plan.

4.4.6. Adverse Events

4.4.6.1. Adverse Event Definitions

Adverse Event - Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of definite, probable, possible, unlikely, or unrelated).

Life-Threatening Adverse Event - Any adverse event that places the participant, in view of the investigator, at immediate risk of death from the reaction.

Serious Adverse Event (SAE) - Any adverse event that results in any of the following outcomes: death, a life threatening adverse event, in-patient hospitalization or prolongation of existing hospitalization, a persistent or significant disability/incapacity, or a congenital anomaly/birth defect.

<u>Unanticipated adverse device effect (UADE)</u> - Any serious adverse effect on health or safety, any life-threatening problem or death caused by, or associated with a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the application; or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects.

Unexpected Adverse Event - Any adverse event, the specificity or severity of which is NOT listed in the study protocol, product inserts or informed consent document.

Attribution - The determination of whether an adverse event is related to a medical treatment or procedure. Attribution categories:
Definite The adverse event is ***clearly related*** to the study drug/device/procedure/ treatment(s).

Probable The adverse event is ***likely related*** to the study drug/device/procedure/ treatment.
For BMT CTN studies: the adverse event is not likely to be caused by the subject's underlying medical condition or other concomitant therapy, and the nature of the adverse event or the temporal relationship between the onset of the adverse event and study drug/device/treatment administration lead the investigator to believe that there is a reasonable chance of causal relationship.

Possible The adverse event ***may be related*** to the study drug/device/procedure/ treatment(s).
For BMT CTN studies: the adverse event could be attributed to the subject's underlying medical condition or other concomitant therapy, but the nature of the adverse event or the temporal relationship between the onset of the adverse event and study drug/device/treatment administration lead the investigator to believe that there could be a causal relationship.

Unlikely The adverse event is ***doubtfully related*** to the study drug/device/ procedure/treatment(s).

Unrelated The adverse event is ***clearly NOT related*** to the study drug/device/procedure/ treatment(s).
For BMT CTN studies: the adverse event is most plausibly explained by the subject's underlying medical condition or other concomitant therapy, or the adverse event has no plausible biological relationship to study drug/device/ treatment.

Common Terminology Criteria Adverse Events (CTCAE) – a descriptive terminology developed by the National Cancer Institute (NCI) for use in reporting adverse events. The CTCAE includes a grading (severity) scale for each adverse event term. Exhibits 6-1-1 and 6-1-2 provide reporting requirements for BMT-related complex/multi-component events. A copy of the current CTCAE guidelines is located at http://ctep.cancer.gov/reporting/.

Grade – Severity of the adverse event. Grades were developed using the following guidelines:

Grade 0 – No adverse event or within normal limits
1 – Mild adverse event
2 – Moderate adverse event
3 – Severe adverse event
4 – Life-Threatening or disabling adverse event
5 – Fatal adverse event

4.4.6.2. Device Effect Reporting

4.4.6.2.1. Adverse Device Effect Reporting

Unanticipated Adverse Device Effects will be reported via a web-based adverse event (AE) system identical to reporting for Unexpected Grade 3-5 adverse events. Any UADE that is a direct result of the CliniMACS CD34 System requires reporting through an expedited AE reporting system within 24 hours of the investigator/clinical site becoming aware of the event. The Adverse Event Coordinator will review daily all submitted unanticipated adverse device effects and forward the information to the Medical Monitor for review. Refer to section 4.5.5.3. for the remainder of reporting procedures. Unanticipated adverse device effects, captured on the AE forms described above, require FDA notification via submission of an IDE safety report. The DCC safety group will submit IDE safety reports regarding all unanticipated adverse device effects within 10 days from initial event notification.

In addition, any SAE assessed by the investigator to be a direct result of the CliniMACS CD34 System requires reporting through an expedited AE reporting system within 24 hours of the investigator/clinical site becoming aware of the event.

4.4.6.2.2. Reporting of Other Device Events

Any device malfunction, device failure, aborted device run, or product contamination must be reported through the device issues form within 24 hours of the investigator/clinical site becoming aware of the event. Though completion of the form is not required until day 7, sites are expected to complete as much information as is available on the form within 24 hours in the event a device malfunction, device failure, aborted device run, or product contamination occurs.

4.4.6.3. Adverse Event Reporting

Unexpected Adverse Events: Unexpected adverse events will be reported via a web-based adverse event (AE) system. The Adverse Event Coordinator will review daily all submitted unexpected adverse events and forward the information to the Medical Monitor for review.

Reporting of patient adverse events (AEs) will be consistent with the BMT CTN Manual of Procedures (MOP). Unexpected, grades 3-5 AEs, irrespective of the attribution of the event to the study drug/device/procedure/treatment, will be reported through an expedited AE reporting system

via the web-based electronic data capture system, AdvantageEDC, and will be graded according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 4.0. Unexpected, grades 4-5AEs must be reported within 24 hours of knowledge of the event. Unexpected, grade 3 AEs must be reported within three business days of knowledge of the event. The NHLBI Data and Safety Monitoring Board will receive summary reports of all adverse experiences at least twice yearly.

All unexpected adverse events will be reviewed by the Medical Monitor within 2 business days of receiving the summary of the adverse event from the transplant center. If the Medical Monitor requires additional information to make his/her assessment, transplant centers will have 4 business days to respond to the request for additional information.

The Medical Monitor has medical expertise relevant to the study protocol and may request the participant's treatment assignment when reviewing the adverse event. A designated person at the DCC is responsible for notifying the Project Officer (National Heart Lung and Blood Institute [NHLBI]) immediately of all Grade 3-5 unexpected adverse events and of any concerns regarding the frequency or type of adverse event(s) on a study or study treatment arm. The NHLBI Project Officer (or designee) is responsible for reviewing the adverse event materials to determine if the materials are complete. If there are any concerns regarding the type or frequency of the event, the NHLBI Project Officer will request that the Data Safety Monitoring Board (DSMB) Executive Secretary notify the DSMB Chair. The DSMB Chair will review the adverse event materials, determine if the information is complete, determine if additional DSMB review is required and make recommendations to the NHLBI concerning continuation of the study. Full documentation of the procedures will be available at the DCC.

The Medical Monitor will review cumulative unexpected grades 3-5 SAEs on a quarterly basis (data will be reported in a blinded fashion). The Medical Monitor may seek additional guidance from one of the DCC Principal Investigators, based on the expertise required, for their assessments as long as the DCC Principal Investigator’s institution is not participating in the protocol under consideration, and the DCC Principal Investigator is not considered to be otherwise in conflict by the NHLBI or by the Steering Committee. If there any concerns regarding safety, the NHLBI Program Directors will be notified immediately. The Medical Monitor will provide a written summary of the safety concern.

The DCC will prepare semi-annual summary reports of all unexpected adverse events for the NHLBI Project Officer and DSMB Chair. Semi-annual reports will be made available on a secure website and the NHLBI Project Officer and DSMB Chair will be notified by e-mail when the materials are posted.

Expected Adverse Events: The DCC will prepare semi-annual summary reports of all Grade 5 expected adverse events for the NHLBI Project Officer and the DSMB Chair. Semi-annual reports will be made available on a secure website and the NHLBI Project Officer and DSMB Chair will be notified by e-mail when the materials are posted. Grade 3-5 expected adverse events defined in the interim analysis plan will be reported as defined in the protocol. Any concern regarding the type or frequency of a Grade 3-5 expected adverse event will be reported to the NHLBI Project

Officer who will determine if referral to the DSMB is warranted. If required, data materials will be provided by the DCC. The DSMB Executive Secretary will arrange for review by the DSMB Chair. The Chair will determine if additional DSMB review is required and make recommendations to the NHLBI concerning continuation of the study. The DCC will ensure that any additional reporting requirements defined by the NHLBI Project Officer, DSMB Chair and other oversight groups are identified and implemented. The DCC in collaboration with the NHLBI Project Officer will determine the exact content of these summary reports and the reporting schedule.

The Protocol Coordinator and Medical Monitor will review the adverse events monitored for stopping guidelines at least monthly.

Additionally, the Protocol Coordinator and Medical Monitor will review events reported on the protocol-specific toxicity form, the GVHD forms and the infection forms on a regular basis (at least semi-annually) to assess whether there are safety concerns that should be referred to the DSMB. The Medical Monitor may seek additional guidance from one of the physicians at the DCC in these assessments as long as this physician's institution is not participating in the protocol under consideration.

TABLE 4.5a: REPORTING UNEXPECTED ADVERSE EVENTS ON A BMT CTN PHASE II OR PHASE III STUDY

SEVERITY GRADE	ATTRIBUTION	TRANSPLANTCENTER REPORTING REQUIREMENTS
5 - Fatal 4 - Life-Threatening or Disabling	All attributions	Submit the adverse event form and a summary of the event to the DCC within 24 hours of the event. For Grade 5, also submit study death form to the DCC. Submit all completed AE forms to the DCC within 4 working days. For Grade 5, the summary should include potential contributing causes of death. Information reported for the adverse event must include: Name of adverse event, date of first onset, peak severity, relationship to study drug/device/procedure/treatment, resolution date, actions taken with respect to administration of study drug/device/procedure/treatment, and other treatment for the adverse event.
3 – Severe	All attributions	Submit the adverse event form and a summary of the event to the DCC within 3 working days of the adverse event. Submit all completed AE forms to DCC within 4 working days
	Definite Probable Possible	Information reported for the adverse event must include: Name of adverse event, date of first onset, peak severity, relationship to study drug/device/procedure/treatment, resolution date, actions taken with respect to administration of study drug/device/procedure/treatment, and other treatment for the adverse event. Multiple recurrences of the same adverse event should be reported separately.
	Unlikely Unrelated	Information reported for the adverse event must include: name of adverse event, date of first onset, peak severity, and relationship to the study drug/device/treatment. Multiple recurrences of the same adverse event should be reported together.

Note: Any adverse event prompting a change in the administration of study drug/device/procedure/treatment must include resolution date, actions taken with respect to administration of study drug/device/procedure/treatment, and other treatment for the adverse event.

TABLE 4.5b: REPORTING EXPECTED ADVERSE EVENTS ON A BMT CTN PHASE II OR PHASE II STUDY

SEVERITY GRADE	ATTRIBUTION	TRANSPLANT CENTER REPORTING REQUIREMENT
5 – Fatal	All attributions	Submit study death form to the DCC within 24 hours of learning of the death. Submit death summaries and/or autopsy reports of the expected adverse event to DCC quarterly or as requested. The summaries should include potential contributing causes of death.
4 – Life-Threatening or disabling	All attributions	Submit study form(s) capturing data on the expected adverse event to the DCC at the form's scheduled due date. If the event is not captured on a study from, report using the AE system in an expedited manner.
3 – Severe	All attributions	Submit study form(s) capturing data on the expected adverse event to the DCC at the form's scheduled due date.

Note: Selected Grade 3-5 events will be tracked and regularly monitored by the DCC and DSMB as specified in protocol-specific monitoring plans.

CHAPTER 5

5. STATISTICAL CONSIDERATIONS

5.1. Study Design and Objectives

The study is designed as a three arm randomized Phase III, multicenter trial comparing two calcineurin inhibitor (CNI)-free strategies for GVHD prophylaxis to one another and to standard tacrolimus and methotrexate (Tac/Mtx) in patients with acute leukemia or myelodysplasia undergoing myeloablative conditioning hematopoietic stem cell transplantation. The target enrollment is 345 patients, 115 for each arm.

5.1.1. Accrual

It is estimated that 42 months of accrual will be necessary to enroll the targeted sample size.

5.1.2. Randomization

Patients will be randomized at a ratio of 1:1:1 between the treatment arms using permuted blocks of random sizes. Randomization will be stratified by donor type (sibling vs. unrelated) and age (<18 vs. 18-40 vs. >40).

5.1.3. Primary Endpoint

The primary endpoint is the chronic GVHD free, relapse free survival (CRFS) probability, treated as a time to event outcome. The primary analysis will be performed using the intent-to-treat principle so that all randomized patients will be included in the analysis. Development of moderate to severe chronic GVHD, relapse, or death will be considered failures for this endpoint, which will be adjudicated by a blinded endpoint review committee.

5.1.4. Primary Hypothesis

There are three primary null hypotheses of the study, for the pairwise comparisons of each of the two CNI-free strategies (CD34 selection and Post-Transplant Cy) against the Tac/Mtx control. Using HR to denote hazard ratios for the CRFS primary endpoint, the hypotheses are

$$H_{01}: \quad HR_{\text{CD34select vs. Tac/Mtx}} = 1 \text{ vs. } H_{a1}: \quad HR_{\text{CD34select vs. Tac/Mtx}} \neq 1 \text{ AND}$$
$$H_{02}: \quad HR_{\text{PostTxCy vs. Tac/Mtx}} = 1 \text{ vs. } H_{a2}: \quad HR_{\text{PostTxCy vs. Tac/Mtx}} \neq 1 \text{ AND}$$
$$H_{03}: \quad HR_{\text{CD34select vs. PostTxCy}} = 1 \text{ vs. } H_{a3}: \quad HR_{\text{CD34select vs. PostTxCy}} \neq 1 \text{ AND}$$

.

These three hypotheses will each be tested at the 0.05 / 3 level using a Bonferroni correction to control the overall type I error rate.

5.1.5. Duration of Follow-Up

All patients will be followed for 2 years post-transplant for primary and secondary endpoints.

5.2. Sample Size and Power Considerations

The primary analysis will be done using log-rank tests for each pairwise comparison, with a type I error of 0.05/3 using a Bonferroni correction to account for multiple testing. The primary analysis is planned to be conducted when 155 events have been observed for each comparison to control. This assumes that the CRFS probabilities in the control group are 46%, 28%, and 22% at 6 months, 1 year, and 2 years, based on CIBMTR data, uses a piecewise exponential survival function to fit those CRFS probabilities, and ensures approximately 85% power to detect a hazard ratio of 0.576 (corresponding to a 20% difference in CRFS at 1 year). No stopping rules for efficacy are planned, because of the interest in having follow up for overall survival on all patients. However, the study design will incorporate interim futility analyses so that one or more of the CNI free arms may be dropped, if the conditional power for the comparison to control under the alternative hypothesis (HR=0.576) is less than 15%. If a treatment group is dropped for futility, the type I error is still maintained at 0.05/3 (see further discussion in section 5.5.1.). We simulated the operating characteristics of this design, using uniform accrual of 345 patients over 42 months, 1 year of follow up on the last patient enrolled (which is the expected time at which the targeted number of events is observed), follow-up censored at 2 years, and an interim futility analysis at 2 years, which is at approximately 57% of the planned accrual and approximately 45% of the expected number of events.

Simulation results are shown in the table below, where groups A and B refer to the CNI-free strategies, and group C refers to the control. All tests in the study are two-sided, and the two-sided familywise type I error rate is shown in the overall power column of the table when both HR are 1.0. These results indicate that the overall type I error rate is controlled at 5% conservatively, primarily due to the impact of the futility analysis which may result in treatment arms being dropped and comparisons not being performed. Note that since the significance level is not adjusted for the futility stopping rule, even if the futility stopping rule is not followed exactly the study will still control the type I error rate. We also looked at type I error for the various pairwise comparisons. The two-sided type I error rate is very low for the CNI-free comparison under the overall null hypothesis (all groups have the same CRFS). This is because frequently one or more of the treatment groups are dropped, preventing this comparison from occurring. The futility analysis also has an impact on the type I error rate for the pairwise comparisons to control, in the sense that the one-sided type I error rate favoring the control is essentially zero due to the futility analysis in place. Therefore, the two-sided type I error rate is approximately equal to the one-sided type I error rate favoring the CNI-free arm. As expected, the simulations also show that the power for each comparison to the control group is maintained at 85%. If a CNI-free approach has no impact on CRFS relative to the control group, there is a 48-49% chance of stopping it early for futility. Note that the power for the CNI-free comparison (A vs. B) is low, but this is mainly due to the fact that often one of the arms is closed early for lack of efficacy, precluding its subsequent comparison.

HR relative to control (A vs. C, B vs. C)	Interim analysis Decision				Power at final analysis			
		Continue with						
	Stop	A, C	B, C	A, B, C	Overall	A vs. C (2-sided ≈1-sided)	B vs. C (2-sided ≈1-sided)	A vs. B (2-sided)
1.0,1.0	29.7%	19.6%	19.7%	31.0%	1.8%	0.9%	0.9%	0.2%
0.576,1.0	0.4%	48.5%	0.1%	50.9%	85.5%	85.2%	1.3%	35.5%
0.576,0.576	0.0%	0.3%	0.4%	99.2%	94.2%	84.7%	84.6%	1.7%

5.3. Interim Analysis and Stopping Guidelines

No formal interim analyses for efficacy will be used. Although the primary endpoint focuses on chronic GVHD through the CRFS endpoint, it is important to get good estimates of the impact of the treatments on a key secondary endpoint of overall survival. Stopping early for efficacy on the primary endpoint would negatively impact the precision of our estimate of the hazard ratio for overall mortality. We also did not include in the design an option for closure of the control group while keeping the two treatment arms open, in the event that at least one of the treatments demonstrate early efficacy. This would also negatively impact the precision of our estimate of the hazard ratio relative to control for a key secondary endpoint of overall mortality. Interim analyses for futility will be conducted at times coincident with regularly scheduled meetings of the NHLBI-appointed Data and Safety Monitoring Board (DSMB), starting when approximately 45-50% of the targeted number of events have been observed. The interim analysis for futility is based on conditional power less than 15% under the targeted alternative hypothesis for the comparisons to the control group. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures. The stopping guidelines serve as a trigger for consultation with the DSMB for additional review and are not formal "stopping rules" that would mandate automatic closure of study enrollment. Toxicity, adverse events, and other safety endpoints will be monitored regularly and reported to the DSMB at each meeting.

5.3.1. Guidelines for Safety Monitoring

Monitoring of a key safety endpoint will be conducted monthly, and if rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures. The stopping guideline serves as trigger for consultation with the DSMB for additional review.

The key safety endpoint for this study is mortality. The rate of mortality will be monitored up to 100 days post-randomization separately in each of the three treatment arms. At least three events must be observed in order to trigger review. The expected probability of 100 day mortality after a myeloablative transplant is 15-20%, based on CIBMTR data. Each month, the null hypothesis that the 100-day mortality rate is less than or equal to 20% is tested. An extension of the sequential probability ratio test (SPRT) for censored exponential data will be used for monitoring, as described in greater detail below and in Appendix G.

This sequential testing procedure conserves type I error at 5% across all of the monthly examinations for a treatment arm. The SPRT can be represented graphically. At each monthly interim analysis, the total time on study in months (x axis) is plotted against the total number of endpoints (y axis) (e.g., patients experiencing death). The continuation region of the SPRT is defined by two parallel lines. Only the upper boundary will be used for monitoring to protect against excessive 100-day mortality. If the graph falls above the upper boundary, the SPRT rejects the null hypothesis, and concludes that there are more events than predicted by the observed time on study. Otherwise, the SPRT continues until enrollment reaches the maximum of 110 patients.

This procedure assumes a censored exponential distribution for the time until death during the first 100 days, and censors follow-up time after 100 days. Only deaths that occur on or before the patient has been followed for 100 days are counted. Total time on study is computed as time from registration to death, or to 100 days, whichever comes first, summed for all patients on study.

The usual measures of performance of an SPRT are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$ and of accepting H_1 when $\theta = \theta_1$, respectively, and the expected sample size $E(N|\theta_i)$. The tests to be used in this protocol were developed from the following SPRT:

> A SPRT contrasting 20% versus 35% 100-day rate of mortality results in decision boundaries with a common slope of 0.096 and an upper intercept of 4.03, with nominal type I and II errors of 6% and 15%, respectively.

The actual operating characteristics of the truncated test, shown in Table 5.3, were determined in a simulation study that assumed uniform accrual of 115 individuals over a 42 month time period, and exponential time to failure after randomization.

TABLE 5.3: OPERATING CHARACTERISTICS OF SEQUENTIAL TESTING PROCEDURE FROM A SIMULATION STUDY WITH 10,000 REPLICATIONS

Day 100 MORTALITY

True 100-Day Rate	20%	30%	35%
Probability Reject Null	0.046	0.669	0.930
Mean Month Stopped	44.0	28.2	18.8
Mean # Endpoints in 100 Days	22.4	21.7	16.6
Mean # Patients Enrolled	111.8	74.6	50.9

For example, the testing procedure rejects the null hypothesis in favor of the alternative 5% of the time when the true 100-day mortality rate is 20%, and 93% of the time when the rate is 35%. This corresponds to a type I error rate of $\alpha = 0.05$ and a type II error rate of $\beta = 0.07$. When the true 100-day mortality rate is 35%, on average, the DSMB will be consulted 19 months after opening, when 17 events have been observed in 51 patients.

5.4. Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Characteristics to be examined are: age, gender, race/ethnicity, performance status, primary disease/disease risk, CMV status, HCT-comorbidity index, time from diagnosis to transplantation, cytogenetic at diagnosis (AML and ALL), HLA matching, and number of regimens prior to transplant (lymphoma only). Between group comparisons will be performed for continuous variables via a Kruskal-Wallis test and for categorical variables, via the chi-square test. A secondary analysis of all outcomes will adjust for age, performance status, primary disease/disease risk, and donor type, in addition to any other demographic and baseline characteristics which are statistically different between treatment arms ($p<0.1$).

5.5. Analysis Plan

5.5.1. Analysis of the Primary Endpoint

The primary outcome of the trial is CRFS, treated as a time to event variable. There are three primary pairwise comparisons between the CNI-free strategies and the Tac/Mtx control group, each to be tested at a Bonferroni adjusted significance level of 0.05/3. We do not use adaptive design strategies to modify the multiplicity adjustment if a treatment group is dropped for futility at an interim analysis. This allows us to directly apply gatekeeping strategies to control the familywise type I error rate also across a key secondary endpoint of overall survival, as described in 5.5.2.2. These comparisons of CRFS will be done using pairwise log-rank tests, applied to the primary intent-to-treat population of all randomized patients. The hazard ratio, along with confidence intervals, will be estimated from a Cox model with treatment as a covariate. Kaplan-Meier estimates of CRFS will also be described for each group, along with confidence intervals at 1 and 2 years. A secondary analysis of CRFS will be conducted using Cox regression, adjusting for donor type, age, performance status, and primary disease/disease risk, in addition to any other demographic and baseline characteristics which are statistically different between treatment arms ($p<0.1$).

5.5.2. Analysis of Secondary Endpoints

Overall survival will be a key secondary endpoint, with an analysis plan which ensures control of the overall type I error rate, as described below. All other secondary endpoints will be considered exploratory without explicit control of the type I error rate, and the analyses of these endpoints will use the transplanted populations rather than the ITT population. All time to event endpoints below will also have secondary analyses using Cox regression, adjusting for donor type, age, performance status, and primary disease/disease status, in addition to any other demographic and baseline characteristics which are statistically different between treatment arms ($p<0.1$). Details of the analyses are provided below.

5.5.2.1. Relapse-Free Survival

Relapse-free survival curves will be estimated using the Kaplan-Meier estimator, and compared between treatment groups using the log-rank test. Hazard ratios, along with confidence intervals, will be estimated from a Cox model with treatment group as a covariate.

5.5.2.2. Overall Survival

Overall survival will be a key secondary endpoint, with explicit control of the type I error rate through a gatekeeper approach. Formal significance testing of OS between a CNI-free strategy and the control will be conducted if the corresponding CRFS comparison is significant. This OS comparison will be done using a Bonferroni adjusted significance level of 0.05/3 to account for three potential CNI-free comparisons to the control. Otherwise, survival analyses will be considered exploratory. Overall survival curves will be estimated using the Kaplan-Meier estimator applied to the ITT population, and compared between treatment groups using the log-rank test. The hazard ratio, along with confidence intervals, will be estimated from a Cox model with treatment group as a covariate. Overall survival will also be described in each arm from the time of transplant.

5.5.2.3. Transplant-Related Mortality

Incidence of TRM will be estimated using the cumulative incidence function, treating relapse/progression as a competing risk. Incidence of TRM will be compared between the treatment arms using Gray's test[71].

5.5.2.4. Immunosuppression-Free Survival

Proportions of patients alive, relapse free, and off immune suppression at one year will be described for each group, and compared using the chi-square test. If there is censoring prior to one year, multistate models will be constructed to estimate these probabilities. Agreement between this endpoint and the primary endpoint of CRFS will be described using cross-tabulation frequencies and assessed using the Kappa statistic.

5.5.2.5. Disease Relapse

Incidence of relapse will be estimated using cumulative incidence function, treating death in remission as a competing risk. Incidence of relapse will be compared between the treatment arms using Gray's test.

5.5.2.6. Hematologic Recovery

Incidence of neutrophil and platelet engraftment from the time of transplant will be estimated using the cumulative incidence function with death prior to engraftment as the competing risk. Incidence of neutrophil engraftment at 28 days and incidence of platelet engraftment at 60 days will be

compared between the treatment arms using a pointwise comparison of the cumulative incidence probabilities.

5.5.2.7. Graft Failure

Primary graft failure will be assessed as a secondary endpoint. The proportion of patients who do not achieve neutrophil recovery to > 500 cells/µL by Day 28 post HSCT will be computed for each treatment arm and compared between the treatment arms using the Z test for binomial proportions. Secondary graft failure will be assessed as a secondary endpoint using cumulative incidence function. The cumulative incidence of secondary graft failure at 2 years post-transplant will be computed along with 95% confidence intervals and compared between the treatment arms using Gray's test. Patients will be considered as reaching the endpoint if the initial neutrophil engraftment is followed by subsequent decline in absolute neutrophil counts < 500 cells/µL, is unresponsive to growth factor therapy, and cannot be explained by disease relapse or medications. Death prior to secondary graft failure will be considered as a competing risk.

5.5.2.8. Acute GVHD of Grades II-IV and III-IV

Cumulative incidence of acute GVHD will be estimated from the time of transplant using the cumulative incidence function, treating death prior to acute GVHD as the competing risk. Cumulative incidence of acute GVHD will be compared between treatment arms using Gray's test.

5.5.2.9. Chronic GVHD

Cumulative incidence of chronic GVHD from the time of transplant will be estimated using the cumulative incidence function, treating death prior to chronic GVHD as the competing risk. Cumulative incidence of chronic GVHD will be compared between treatment arms using Gray's test.

5.5.2.10. Chronic GVHD-Free Survival

Chronic GVHD-free survival curves will be estimated using the Kaplan-Meier estimator, and compared between treatment groups using the log-rank test. Hazard ratios, along with confidence intervals, will be estimated from a Cox model with treatment group as a covariate.

5.5.2.11. Incidence of Toxicities Grade ≥ 3 per CTCAE Version 4.0

All Grade ≥ 3 toxicities will be tabulated by grade for each treatment arm, by type of toxicity as well as the peak grade overall. Toxicity frequencies will be described for each time interval as well as cumulative over time.

5.5.2.12. Incidence of Infections

The number of infections and the number of patients experiencing infections will be tabulated by type of infection, severity, and time period after transplant. The cumulative incidence of severe,

life-threatening, or fatal infections, treating death as a competing event, will be compared between the treatment arms using Gray's test.

5.5.2.13. Immune Reconstitution

It is proposed that quantitative assessment of peripheral blood CD3, CD4, CD8, CD19 and CD56 positive lymphocytes will be performed for each randomized treatment arm at the specified time points assessed after transplant (days 35, 100, 180 and 365). Analysis of effector and regulatory T-cell subsets will also be described in the sub-cohort of adult patients sending samples to a centralized lab. Correlations among the lymphocyte populations will be explored using rank correlations. Analysis will be done separately for each immune reconstitution measurement using a Bonferroni adjusted significance level (0.05/7). Log transformations will be considered to induce normality in the lymphocyte subset counts. Partly conditional regression conditioning on being alive at each time point will be implemented using GEE to compare the longitudinal immune reconstitution measurements over time between the treatment groups[72] . Interactions with time will be tested for and if significant, treatment effects will be estimated separately for each time point. The missing data pattern of the immune reconstitution lab measurements will be examined using graphical techniques and logistic regression models conditional on survival, and if necessary inverse probability of censoring weighted GEE[73] will be used to account for missing data. We anticipate at least 50 samples per treatment arm being available at each time point, even in the adult sub-cohort, based on anticipated 63% overall survival at one year. This sample size will yield at least 85% power to detect a 2 fold increase in cell counts between treatment arms, assuming a 0.95 SD in the log cell counts, a value consistent with our preliminary data. Additional lymphocyte subsets will also be analyzed in the adult sub-cohort in an exploratory fashion using similar methods, except that a false discovery rate controlling procedure will be used to account for multiple testing.[74] In addition to comparing immune reconstitution among the three groups, we will also examine how changes in lymphocyte subpopulations are associated with subsequent clinical outcomes, including development of acute and chronic GVHD, survival, CRFS, relapse, and infection. Cox multivariate regression will be performed using landmark analysis or time dependent covariates to model the relationship between the most recent cell count or change in cell count and the development of subsequent events.

5.5.2.14. Health-Related Quality of life

HQL will be measured at Baseline and then at Days 100 180, 365, and 730 post-transplant using six instruments: the SF36 (both PCS and MCS), FACT-BMT, and MDASI for English and Spanish speaking adult patients (> 18 years), and the PedsQL™ Stem Cell Transplant Module for English speaking pediatric patients (≥ 8 years through 18 years). HQL at each time point will be summarized using simple descriptive statistics (mean, SD). Analysis will be done separately for each instrument using a Bonferroni adjusted significance level (0.05/4). All models will be adjusted for baseline HQL. Partly conditional regression conditioning on being alive at each time point will be used to compare the longitudinal HQL measurements over time between the treatment groups.[72] Interactions with time will be tested for and if significant, treatment effects will be estimated separately for each time point. The missing data pattern of the HQL measurements will be examined using graphical techniques and logistic regression models conditional on survival. At

each time point, estimates of the difference in HQL between the treatments conditional on survival at that time point will be obtained using inverse probability of censoring weighted GEE with independent estimating equations[73] to account for missing data.

5.5.2.15. Subgroup Analysis

Subgroup analyses will be conducted for CRFS according to disease, disease risk and age. Interaction tests between treatment group and subgroup will be conducted within a Cox proportional hazards regression model with treatment, subgroup, and a treatment*subgroup interaction term. A Bonferroni adjusted significance level of 0.05/3=0.0167 will be used for each interaction test to account for multiple testing. If a significant interaction is identified, plots of Kaplan-Meier estimates of CRFS by treatment will be shown separately for each level of the subgroup, and a forest plot will be used to show the HR's for each subgroup.

CHAPTER 6

6. ETHICS AND REGULATORY

6.1. Good Clinical Practice Guidelines

The DCC and the clinical investigators assure that the clinical study is performed in accordance with the protocol, the ICH Harmonized Tripartite Guidelines for Good Clinical Practice 1996, the Declaration of Helsinki (Recommendations guiding physicians in Biomedical Research involving Human Subjects, Helsinki 1964, amended Tokyo 1975, Venice 1983, Hong Kong 1989, Somerset West 1996, Edinburgh, 2000, Seoul 2008, Fortaleza 2013) and applicable regulatory requirements.

6.1.1. Patient Consent

Candidates for the study will be identified as described in Chapter 4 of the protocol. The Principal Investigator or his/her designee at each transplant center will contact the candidates, provide the patient with information about the purpose of the study and obtain consent. The BMT CTN will provide a template of the consent form to each center. Each center will customize the template according to their local requirements and submit it for review by the local Internal Review Board (IRB). The DCC will verify the adequacy of the consent forms prior to submission to the IRB. Each center must provide evidence of IRB approval.

6.1.2. Patient Withdrawal

6.1.2.1. Patient Withdrawal from Intervention

Participation in the trial is voluntary. Patients will be advised that they may voluntarily withdraw from the study at any time and will be instructed to notify the investigator. Patients may choose to withdraw for any reason(s). Patients are not obligated to reveal their reason(s) for withdrawal to the sponsor.

6.1.2.2. Patient Withdrawal from Data Collection

If a patient explicitly states they do not wish to contribute further data to the trial their decision must be respected and notified to the sponsor in writing. In this event details should be recorded in the patient's hospital records, no further CRFs must be completed and no further data sent to the sponsor.

6.1.2.3. Patient Removal from Study

The investigator has the right to terminate the participation of any subject at any time, if s/he deems it in the participant's best interest. The reason and circumstances for study discontinuation will be documented by the site and sent to the DCC.
Reasons for study discontinuation might be:

- Intolerable side effects of the study product.
- Changes in medical status of the patient such that the Investigator believes that patient safety will be compromised or that it would be in the best interest of the patient to stop treatment.
- Pregnancy.
- Withdrawal of consent.
- Relevant non-compliance with the protocol.

6.1.3. Confidentiality

Confidentiality will be maintained by individual names being masked and assigned a patient identifier code upon enrollment in AdvantageEDC. The code relaying the patient's identity with the ID code will be kept separately at the center.

6.1.4. Participation of Women and Minorities

Women and ethnic minorities and other populations will be included in this study. Accrual of women and minorities at each center will be monitored to determine whether their rates of enrollment are reflective of the distribution of potentially eligible women and minorities expected from data reported to the CIBMTR and from published data on incidence of acute leukemia and myelodysplasia in these groups. Centers will be notified if their rates differ significantly from those expected and asked to develop appropriate recruitment reports.

6.1.5. NMDP Unrelated Donors

The National Marrow Donor Program (NMDP) IRB will be responsible for the review and continuing oversight of protocol procedures that relate only to NMDP unrelated donors.

6.1.6. International Unrelated Donor Consent

Donor research consent may be waived for international donors if the country of the donor registry does not consider such donors to be research subjects. However, the donor must meet all donor-eligibility criteria for the study and must consent to donation as per requirements of the collecting donor registry and regulations of the country of donation.

6.2. Protocol Amendments

All protocol amendments containing substantive changes must be reviewed and accepted by members of the DSMB before distribution to Clinical Centers. The review may take place at a DSMB meeting, on an arranged conference call, or by ballot.

Once approved by the DSMB, participating centers (and the FDA if applicable) will be notified of approved protocol amendments by an e-mail announcement from the DCC. Amendment documents may be included with the e-mail announcement and will be posted on the BMT CTN website. The documents will include:

1. A summary of protocol changes
2. A revised protocol with changes highlighted
3. A second version with the changes fully incorporated into the document
4. A rationale for changes may be provided

Other types of amendments containing non-substantive changes may be released to Centers before DSMB notification or deliberation. These changes will be reviewed at the next regularly scheduled DSMB meeting or conference call.

The DCC PIs will determine the appropriate review process in collaboration with the NHLBI Project Officer.

Clinical Centers are responsible for implementing all amendments according to institutional policy.

6.3. Protocol Deviations

Clinical centers should inform the DCC of any major protocol deviations. These will be submitted to the DSMB on a semi-annual basis.

6.4. Premature Termination of the Trial

The sponsor has the right to discontinue the study due to relevant medical or administrative reasons. Participants who still receive medication during the time of discontinuation will undergo a final visit which has to be documented in the CRF.

Possible reasons for discontinuation by the sponsor are:

- failure in recruiting participants,
- data quality is insufficient,
- unforeseen circumstances at the study site that make the continuation of the study impossible,
- early prove of superiority or non-inferiority of a treatment group,
- occurrence of unjustifiable risks or toxicity,
- new scientific knowledge that does not justify continuation of the clinical study.

6.5. Publication Policy

Manuscripts reporting the results of this trial will be prepared and submitted to a reputable peer-reviewed medical journal in accordance with basic ethical principles, including preservation of the accuracy of the results and making both positive and negative results publicly available. In all publications the confidentiality of patients' data will be ensured.

Data analysis and authorship will be conducted according to the BMT CTN MOP. No clinical trial results are released, presented or published without approval from the Publications Committee, the BMT CTN DCC, NHLBI and NCI.

The study is registered in the clinical trials database (www.clinicaltrials.gov) which is accessible to the public. The ClinicalTrials.gov number NCT02345850 allocated to this trial will be quoted in any publications resulting from this trial.

By signing this study protocol, the investigators accept that the results of this clinical trial can be presented to national and international authorities. They also accept that in this context their name, address, qualification and grade of involvement in this trial will be published.

6.6. German Transfusion Law

To comply with the German Transfusion Law requirements, German centers must ensure that the information and documents relating to the traceability for all the stages from donation of the HSC units to their infusion into the recipient (including information about the blood product) are archived in safe custody for a minimum of 30 years, even if the patient withdraws consent.

APPENDIX A

LIST OF ABBREVIATIONS

LIST OF ABBREVIATIONS

7-AAD – 7-amino-actinomycin D
ACD – Acid Citrate Dextrose
AdvantageEDC^{SM} – Proprietary electronic data capture system
AE – Adverse Event
AIBW – Adjusted Ideal Body Weight
ALL – Acute Lymphoblastic Leukemia
ALT – Alanine Aminotransferase
AML – Acute Myeloid Leukemia
ANC – Absolute Neutrophil Count
AST – Aspartate Aminotransferase
ATG – Anti-Thymocyte Globulin
ATP – Adenoside Triphosphate
AUC – Area Under the Curve
B-ALL – Acute B Lymphoblastic Leukemia
BID – bis in die (two times/day)
BM – Bone Marrow
BMT – Bone Marrow Transplant
BMT CTN – Blood and Marrow Transplant Clinical Trials Network
BSA – Body Surface Area
CBC – Complete Blood Count
CCr – Creatinine Clearance Rate
CCR7 – C-C Chemokine Receptor 7
CD127 – Cluster of Differentiation 127
CD16 – Cluster of Differentiation 16
CD19 – Cluster of Differentiation 19
CD25 – Cluster of Differentiation 25
CD27 – Cluster of Differentiation 27
CD3 – Cluster of Differentiation 3
CD31 – Cluster of Differentiation 31
CD34 – Cluster of Differentiation 34
CD38 – Cluster of Differentiation 38
CD4 – Cluster of Differentiation 4
CD45RA - Cluster of Differentiation 45 Antibody
CD56 – Cluster of Differentiation 56
CD8 – Cluster of Differentiation 8
CD95 – Cluster of Differentiation 95
CDC – Centers for Disease Control
cGVHD – Chronic Graft Versus Host Disease
cGY - Centigray
CI – Confidence Interval
CIBMTR – Center for International Blood and Marrow Transplant Research
CMV - Cytomegalovirus
CNI – Calcineurin Inhibitor
CNS – Central Nervous System
CR - Complete Remission
CrCl – Creatinine Clearance
CRF – Case Report Form
CRFS – Chronic GVHD/Relapse-Free Survival
CsA/CSA – Cyclosporine A
CT – Computed Tomography
CTD – Connective Tissue Disease
CTL – Cytotoxic T-Lymphocyte
CTCAE – Common Terminology Criteria for Adverse Events
CY - Cyclophosphamide
DCC – Data and Coordinating Center
DLCO – Diffusing Capacity of the Lung for Carbon Monoxide
DLI – Donor Lymphocyte Infusion
DNA – Deoxyribonucleic Acid
DSMB – Data Safety Monitoring Board
dUCB – Double Umbilical Cord Blood
EBER – Epstein-Barr virus-encoded small RNA
EBV – Epstein Barr Virus
ECG – Electrocardiogram
eCRF – Electronic Case Report Form
EDTA – Ethylenediaminetetraacetic Acid
EFS – Event-Free Survival
EKG - Electrocardiogram
ELISPOT – Enzyme-Linked ImmunoSpot
FACT-BMT – Functional Assessment of Cancer Therapy – Bone Marrow Transplant
FACT-G – Functional Assessment of Cancer Therapy – General Core Questionnaire
FACT-JACIE – Foundation for the Accreditation of Cellular Therapy - Joint Accreditation Committee ISCT-EBMT
FDA – Food and Drug Administration

FEV1 – Forced Expiratory Volume 1
FISH – Fluorescence In Situ Hybridization
FK-506 - Tacrolimus
FKBP12 – FK506 Binding Protein 12
FVC – Forced Vital Capacity
G-CSF – Granulocyte Colony Stimulating Factor
GEE – Generalized Estimating Equation
GFR – Glomerular Filtration Rate
GI - Gastrointestinal
GINA – Genetic Information Nondiscrimination Act
GVHD – Graft versus Host Disease
GVL – Graft Versus Leukemia
HCG – Human Chorionic Gonadotropin
HCT-CI – Hematopoietic Cell Transplant-Specific Co-Morbidity Index
HepA – Hepatitis A
HepB – Hepatitis B
HepC – Hepatitis C
HHV-6 – Human Herpesvirus 6
HIPAA – Health Insurance Portability and Accountability Act
HIV – Human Immunodeficiency Virus
HLA – Human Leukocyte Antigen
HPLC – High Performance Liquid Chromatography
HQL – Health-related Quality of Life
HR – Hazard Ratio
HSCT – Hematopoietic Stem Cell Transplant
HSV – Herpes Simplex Virus
HTLV – Human T-Lymphotrophic Virus
IBW – Ideal Body Weight
ICH – International Conference on Harmonisation of Tehcnical Requirements for Registration of Pharmaceuticals for Human Use
IDE – Investigational Device Exemption
IgA – Immunoglobulin A
IgD – Immunoglobulin D
IgG – Immunoglobulin G
IgM – Immunoglobulin M
IL-2Rα – Interleukin 2 Receptor, Alpha
IL-7R – Interleukin 7 Receptor
IPS – Idiopathic Pneumonia Syndrome
IRB – Institutional Review Board
ISCT-EBMT – International Society for Cellular Therapy – European Group for Blood and Marrow Transplantation
ITT – Intention To Treat
IU – International Units
IUD – Intrauterine Device
IV - Intravenous
IVIG – Intravenous Immune Globulin
LDH – Lactate Dehydrogenase
LMP – Latent Membrane Protein
LN – Liquid Nitrogen
LVEF – Left Ventricular Ejection Fraction
MAC – Myeloablative Conditioning
MCS – Mental Component Summary
MDACC – MD Anderson Cancer Center
MDASI – MD Anderson Symptom Inventory
MDS – Myelodysplastic Syndrome
MESNA – Sodium-2-Mercaptoethane Sulphonate
MI – Myocardial Infarction
MMF – Mycophenolate Mofetil
MOP – Manual of Procedures
MOS SF-36 – Medical Outcomes Study Short Form 36
MRD – Minimal Residual Disease
MSKCC – Memorial Sloan Kettering Cancer Center
MTX – Methotrexate
MUD – Matched Unrelated Donor
MUGA – Multi Gated Acquisition Scan
NAAT – Nucleic Acid Amplified Testing
NCBI – National Center for Biotechnology Information
NCI – National Cancer Institute
NHLBI – National Heart, Lung, and Blood Institute
NIH – National Institutes of Health
NK – Natural Killer
NMDP – National Marrow Donor Program
NRM – Non-Relapse Mortality
OHRP – Office for Human Research Protections

OS – Overall Survival
PBMTC – Pediatric Blood and Marrow Transplant Consortium
PBSC – Peripheral Blood Stem Cell
PBMC – Peripheral Blood Mononuclear Cell
PCR – Polymerase Chain Reaction
PCS – Physical Component Summary
PECAM1 – Platelet/Endothelial Cell Adhesion Molecule 1
PET – Positron Emission Tomography
PFT – Pulmonary Function Test
PI – Principal Investigator
PO – Per Os (by mouth)
PR – Partial Response
PTCy – Post-Transplant Cyclophosphamide
PTLD – Post-Transplant Lymphoproliferative Disorder
RA – Rheumatoid Arthritis
RACE – Rapid Amplification of cDNA Ends
rATG – Rabbit Anti-thymocyte Globulin
RBC – Red Blood Cell
RFS – Relapse-Free Survival
RIC – Reduced Intensity ConditioningSCTOD – Stem Cell Therapeutic Outcomes Database
SD – Standard Deviation
SLE – Systemic Lupus Erythematosus
SOP – Standard Operation Procedure
SOS – Sinusoidal Obstruction Syndrome
SPRT – Sequential Probability Ratio Test
T-ALL – T-cell Acute Lymphoblastic Leukemia
Tandem MS – Tandem Mass Spectrometry
TBI – Total Body Irradiation
TCD – T-cell Depleted
TCRαβ – T-cell Receptor Alpha Beta
TID – ter in die (three times/day)
TREC – T-cell Receptor Rearrangement Excision Circle
TRM – Transplant-Related Mortality
ULN – Upper Limit of Normal
VOD – Veno-occlusive Disease
VZV – Varicella Zoster Virus
WBC – White Blood Cell
WHO – World Health Organization

APPENDIX B-1

PATIENT INFORMED CONSENT

Informed Consent to Participate in Research

Your Name: _____

Study Title: **A Randomized, Multi-Center, Phase III Trial of Calcineurin Inhibitor-Free Interventions for Prevention of Graft-versus Host-Disease**

Protocol: BMT CTN # 1301

Principal Investigator: *Insert local PI information*

Sponsor: The National Institutes of Health (NIH) is sponsoring this study by providing financial support through the Blood and Marrow Transplant Clinical Trials Network (BMT CTN).

1. Introduction

We invite you to join this clinical trial, also known as a research study. We are doing this study because we want to compare three transplant procedures to see which is better at preventing Graft-versus-Host Disease (GVHD). You are being asked to join this study because:

1. You have a disease that can be treated by an allogeneic blood or marrow stem cell transplant; and
2. Your doctor plans on using a standard intensity conditioning regimen for your transplant.

This study will take at least two (2) years and will include 345 participants – 115 participants in each of three (3) treatment groups.

This Consent Form will tell you about the purpose of the study, the possible risks and benefits, other options available to you, and your rights as a participant in the study.

Everyone who takes part in research at [*insert facility name*] should know that:

- Being in any research study is voluntary.
- You may or may not benefit from being in the study. Knowledge we gain from this study may benefit others.
- If you join the study, you can quit the study at any time.
- If you decide to quit the study, it will not affect your care at [insert name of facility or institution].
- Please ask the study staff questions about anything that you do not understand, or if you would like to have more information.
- You can ask questions now or any time during the study.
- Please take the time you need to talk about the study with your doctor, study staff, and your family and friends. It is your decision to be in the study. If you decide to join, please sign and date the end of the Consent Form.

You and your doctor will discuss other treatment choices if you do not want to participate in this study.

2. Study Background

The National Institutes of Health (NIH), through the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), are providing staff support and money for this research study. The BMT CTN and the NIH will make decisions about how to manage the study. Miltenyi Biotec, a company that produces a device used to process the stem cells before administering in patients, is also supporting this study with supplies and money.

A hematopoietic stem cell transplant (HSCT) is a standard therapy for blood cancers such as acute leukemias and myelodysplastic disorders. A common problem that may occur after HSCT is a condition known as Graft-Versus-Host Disease (GVHD). The word “graft” refers to the donor blood cells that you will receive during your transplant. The word “host” refers to the person (in this case, you) receiving the cells. GVHD is a complication where the donor graft attacks and damages some of your (the transplant recipient's) tissues. It has two basic forms, an acute form which tends to occur rapidly and is most common in the first three months after the transplant and a chronic form, which develops slowly and at a later time after transplant.

- GVHD can cause skin rash, intestinal problems such as nausea, vomiting, or diarrhea,
- It may also damage your liver and cause hepatitis or jaundice.
- GVHD may also increase your risks of infection.
- Chronic GVHD can affect many organs and causes significant impact on the quality of life of patients.

3. Study Purpose

We are inviting you to take part in this study because you have acute leukemia or myelodysplasia, and a hematopoietic stem cell transplant is a treatment option.

The purpose of this study is to compare three different combinations of treatment plans to see whether one or more of them are better than a standard transplant procedure. The procedures being studied have the objective to reduce the occurrence of chronic GVHD. The procedures included in this clinical trial are:

Treatment Group A: CD34 Selected Peripheral Blood Stem Cell Transplant

Treatment Group B: Bone Marrow Transplant followed by Post-Transplant Cyclophosphamide

Treatment Group C: Bone Marrow Transplant with Tacrolimus and Methotrexate as GVHD Prevention

Doctors primarily want to compare Groups A and B with Group C (control). The study will help doctors make choices about transplants procedures with fewer chronic GVHD complications for patients.

4. Right to Ask Questions and/or Withdraw

You have the right to ask questions about the study at any time. If you have questions about your rights as a participant or you want to leave the study, please contact:
[*insert contact info*]

Being in this study is voluntary. You can choose not to be in this study or leave this study at any time. If you choose not to take part or leave this study, it will not affect your regular medical care in any way.

Your study doctor and study staff will be available to answer any questions that you may have about taking part in or leaving this study.

5. Study Treatment and Tests

We will check your health before you start treatment, while you receive treatment, and for two years after transplant.

<u>Before You Begin the Study</u>
Before you begin the study, you will need to have several exams, tests or procedures to find out if you can be in the study. All patients participating in this study need to have a matched donor. Most of these exams, tests or procedures are part of regular cancer care and may be done even if you do not join the study. These include:

- Medical history
- Physical examination, including height and weight
- Blood and urine tests
- Heart function tests, including EKG and ejection fraction
- Lung (pulmonary) function tests
- Tests to evaluate your cancer, including a bone marrow aspirate/biopsy
- Chest X-ray or chest CT
- A pregnancy test if you are a woman able to have children. If you are pregnant, you will not be able to take part in this study.

If you join the study, the study-specific assessments listed below will be done before you begin the conditioning regimen:

- Health quality of life questionnaires (for English and Spanish speaking adult patients ≥ 18 years of age and English speaking pediatric patients, ages 8 to 18 years).
- *Optional* blood samples for future research (see Section 18: Blood Samples for Future Research)

Study Participation
If you decide to join the study, your participation will last for **2 years** after your transplant. We will ask you to sign this Consent Form and you will get a copy of the signed form to keep.

Randomization
We will use a computer to randomly assign you to 1 of 3 treatment groups. You will have an equal chance of being placed in 1 of the 3 groups. Neither you nor your doctor or study investigator will have any control over which treatment group you will be assigned.

During Your Transplant
The treatments that are used to prevent GVHD either start before or after the infusion of stem cells. These treatments are a combination of immune suppressing drugs and a standard component of the transplant.

The 3 treatment groups being included in this study are outlined below:

Treatment Group A: CD34 Selected Peripheral Blood Stem Cell Graft

- Conditioning regimen: your doctor will select one of two conditioning regimens that are allowed in this treatment group. One includes total body irradiation plus chemotherapy, and the other includes chemotherapy alone.
- The donor graft will be peripheral blood stem cells.
- The donor graft will be processed through a device that removes cells that are associated with the development of GVHD.

Treatment Group B: Post-Transplant Cyclophosphamide

- Conditioning regimen: your doctor will choose one of three conditioning regimens allowed in this treatment group.
- The donor graft will be bone marrow.
- After your transplant:
  - Cyclophosphamide will be given by intravenous infusion (through your vein), over 1-2 hours, on Day 3 and Day 4 after your transplant.

Treatment Group C: Tacrolimus and Methotrexate

- Conditioning regimen: your doctor will choose one of four conditioning regimens allowed in this treatment group.
- Before your transplant:
  - Tacrolimus will be given as a pill by mouth or by intravenous infusion (through your vein) twice a day, beginning three (3) days before your transplant. The amount of drug given will slowly be decreased and eventually stopped. This process occurs over several months.
- The donor graft will be bone marrow.
- After your transplant:
  - Methotrexate will be given by intravenous infusion (through your vein) on four (4) different days (1, 3, 6 and 11) after your transplant.

Both Treatment Groups A and B do not require long term use of medication to suppress the immune system.

<u>Peripheral Blood Stem Cell or Bone Marrow Transplant</u>
On your transplant day, the bone marrow or peripheral blood stem cells will be given to you through your catheter, like a blood transfusion. The cells will travel to your bone marrow where they will start to make healthy, new blood cells after several weeks.

<u>Health Evaluations After the Transplant</u>
We will test (evaluate) your health during the study. These tests and how often they are scheduled are standard care for patients receiving an allogeneic transplant. Most of these would be done even if you were not part of this study. You will be watched closely for any signs and symptoms of GVHD.

- Physical exam to assess toxicities, and infections weekly until Day 63 and then at Days 100, 150, 180, 270, 365 and 730.
- Physical exam to assess GVHD weekly starting Day 7 until Day 63 and then at Days 100, 150, 180, 270, 365 and 730.
- Routine blood tests (cell counts, liver and kidney function) weekly until Day 63 and then at Days 100, 180, 270, and 365.

- Blood tests to monitor for CMV and EBV weekly until Day 100 and then at each clinical assessment until Day 180.
- Restaging tests to see how much cancer you have after transplant on Days 100, 180, 365 and 730.
- Health quality of life questionnaires after the transplant on Days 100, 180, 365 and 730 (for English and Spanish speaking adult patients ≥ 18 years of age and English speaking pediatric patients, ages 8-18 years).
- *Optional* blood samples for future research after transplant on Days 35, 100, 180 and 365 (see Section 18: Blood Samples for Future Research).

6. Health Quality of Life (for English and Spanish speaking adult patients, and English speaking pediatric patients only)

We will ask you about your general health and how well you feel while you participate in this study. Even though different treatments may treat a disease equally well, there might be a difference in how patients feel or the side effects they have after their treatment. This is important information for when we evaluate the treatments in this study.

We will collect information by using surveys. The surveys will ask about:

- How you feel
- What symptoms you might have and how they affect you
- How well can you do regular daily activities

You will need to fill out the surveys and each survey should take about 30 minutes to finish. Your answers will help us understand how your transplant treatment affects how you feel, what you can do, and your general quality of life.

7. Risks and Discomforts

You will have side effects while on the study. Side effects can range from mild to serious. The risks and discomforts of participating in this study will be similar to what you may have with stem cell transplant if you do not participate in this study, but you might do better or worse than on standard transplant treatment. Your health care team may give you medicines to help lessen side effects such as feeling sick to your stomach (nausea) among other support treatments. In some cases, side effects can be long lasting or may never go away.

<u>Risks and Toxicities Related to Conditioning Regimens</u>
The table below describes all conditioning regimens that are allowed to be used in this clinical trial. The regimen you will receive depends on the treatment group you will be assigned and your doctor's choice. Some of these regimens are used in transplants performed outside a clinical trial.

TABLE 1: CONDITIONING REGIMEN OPTIONS BY TREATMENT GROUP

Treatment Group A: CD34 Selection		Treatment Groups B &C: PTCy & Control	
1	**Total Body Irradiation / Cyclophosphamide/Thiotepa/AntiThymocyte Globulin (ATG)**	**3**	**Busulfan/Cyclophosphamide (Bu/Cy)**
		4	**Busulfan /Fludarabine (Bu/Flu)**
2	**Busulfan/Melphalan/Fludarabine/ AntiThymocyte Globulin (ATG)**	**5**	**Cyclophosphamide/Total Body Irradiation (Cy/TBI)**
		6	**Total Body Irradiation/Etoposide (TBI/Etoposide) ONLY FOR THE CONTROL ARM**

The risks associated with each medication and or radiation you will receive as part of the conditioning regimen are listed below. The expected frequency of each of these side effects is shown in Table 2.

TABLE 2 - RISKS AND SIDE EFFECTS

Likely	What it means: This type of side effect is expected to occur in more than 20% of patients. This means that 21 or more patients out of 100 might get this side effect.
Less Likely	What it means: This type of side effect is expected to occur in 20% of patients or fewer. This means that 20 patients or fewer out of 100 might get this side effect.
Rare, but Serious	What it means: This type of side effect does not occur very often – in fewer than 2% of patients – but is serious when it occurs. This means that 1 or 2 patients (or fewer) out of 100 might get this side effect.

TABLE 3 – ADVERSE EVENTS

Busulfan

Likely Side Effects (May happen in more than 20% of patients)	**Less Likely** (May happen in less than 20% of patients)	**Rare** (May happen in less than 2% of patients)
Abdominal discomfort Constipation Diarrhea Dizziness Fluid retention Headache Heartburn Insomnia Lack of appetite Mouth sores Nausea and vomiting Running nose Skin rashes Irregular or no menstrual cycles Tachycardia	Cough Hepatic Veno-occlusive disease High blood pressure High magnesium and phosphorus levels in the blood High sugar levels in the blood Infertility Low blood pressure Seizures Shortness of breath	Cataracts Lung fibrosis

Cyclophosphamide

Likely Side Effects (May happen in more than 20% of patients)	**Less Likely** (May happen in less than 20% of patients)	**Rare** (May happen in less than 2% of patients)
Sores in mouth or on lips Damage to male (tests) and female (ovaries) sex glands Diarrhea Fluid retention Hair loss Infertility Irregular or no menstrual cycles Loss of appetite Nausea, Vomiting Suppression of the immune system Decreased platelet count and increased risk of bleeding	Bleeding in the bladder Anemia (low red blood cell count) Damage to the fetus if you become pregnant while taking drug Stomach pain Skin rash	Allergic reaction Lung fibrosis (scarring of lung tissue with cough and shortness of breath) Serious skin rashes Severe heart muscle injury and death (at very high doses) Secondary (new) cancers

If you are taking cyclophosphamide, your doctor may also prescribe you a medicine called **Mesna**. Mesna helps prevent bladder discomfort and bleeding that can occur from taking cyclophosphamide.

Etoposide

Likely Side Effects (May happen in more than 20% of patients)	**Less Likely** (May happen in less than 20% of patients)	**Rare** (May happen in less than 2% of patients)
Diarrhea Hair loss Nausea and vomiting	Mucositis Constipation Abdominal pain	Allergic reaction Peripheral Neuropathy

Fludarabine

Likely Side Effects (May happen in more than 20% of patients)	**Less Likely** (May happen in less than 20% of patients)	**Rare** (May happen in less than 2% of patients)
Diarrhea Mouth sores Nausea and vomiting Suppression of the immune system	Fever Numbness in the extremities Sleepiness Visual changes Weakness	Coma Cough Inflammation of the lung Interstitial Pneumonia Skin rash

Melphalan

Likely Side Effects (May happen in more than 20% of patients)	**Less Likely** (May happen in less than 20% of patients)	**Rare** (May happen in less than 2% of patients)
Constipation Diarrhea Hair loss Mucositis Nausea and vomiting	Heart rhythm abnormalities Hepatitis Kidney failure	Allergic reaction Interstitial Pneumonia Seizure Lung fibrosis

Total body Irradiation (TBI)

Likely Side Effects (May happen in more than 20% of patients)	**Less Likely** (May happen in less than 20% of patients)	**Rare** (May happen in less than 2% of patients)
Diarrhea (loose stools) Nausea (sick to the stomach) Stomach cramps Vomiting (throwing up) Painful swelling of the parotid gland (salivary glands under the ears) for a few days Short-Term hair loss Anemia Infection Bleeding Cataracts Sterility (inability to have children Growth failure Endocrinopathies (such as thyroid disease or diabetes) Mouth sores	Lung inflammation Pneumonia Redness of the skin Liver problems	Risk of developing other cancers in the future as a consequence of having received the total body irradiation Difficulty swallowing Back problems Kidney problems

Thiotepa

Likely Side Effects (May happen in more than 20% of patients)	**Less Likely** (May happen in less than 20% of patients)	**Rare** (May happen in less than 2% of patients)
Lower white blood cell count with increased risk of infection Diarrhea (loose stools) Vomiting (throwing up) Liver damage Lower sperm production in men Hair loss Nausea (feeling sick to your stomach) Loss of appetite Missing or stopping menstrual cycle in women Mouth/throat sores Sterility (inability to have children)	Liver abnormalities Skin rash Change in skin coloring Risk of bleeding due to low platelet count	Confusion Disorientation

Rabbit Anti-Thymocyte Globulin (rATG)

Likely Side Effects (May happen in more than 20% of patients)	**Less Likely** (May happen in less than 20% of patients)	**Rare** (May happen in less than 2% of patients)
Fever Shaking chills Low blood pressure Skin rash Itching Decreased platelet counts Decreased white blood cell counts	Serum sickness, consisting of : -Severe skin rashes -Mouth sores -Vaginal sores -Pain/swelling of joints -Kidney damage	Severe allergic reaction which may cause: -Life-Threatening drop in blood pressure -Wheezing -Difficulty breathing -Severe hives

Risks and Toxicities Related to GVHD Prophylaxis

If you were assigned to the CD34 Selection Arm and your cells are selected with the CliniMACS® CD34 Reagent System, you may receive low doses of iron, iron-dextran and monoclonal antibody when the selected cells are re-infused. Our experience shows that these low doses are unlikely to cause any bad side effects, including cancer.

If you were assigned to the control group (treatment group C) you will receive medications to help prevent the development of GVHD.

Methotrexate

Likely Side Effects (May happen in more than 20% of patients)	**Less Likely** (May happen in less than 20% of patients)	**Rare** (May happen in less than 2% of patients)
Decreased white blood cell count with increased risk of infection. Fatigue Infections	Nausea/Vomiting Irritation or sores in the lining of the throat or mouth Diarrhea Abdominal discomfort Fever Chills Anemia Abnormal liver tests Kidney failure	Dizziness Scarring of the lungs

Tacrolimus (FK506, Prograf®)

Likely Side Effects (May happen in more than 20% of patients)	**Less Likely** (May happen in less than 20% of patients)	**Rare** (May happen in less than 2% of patients)
▪ Kidney problems ▪ Loss of magnesium, calcium, potassium ▪ High blood pressure ▪ Tremors ▪ Increases in cholesterol and triglyceride ▪ Decreased platelet count with increased risk of bleeding ▪ Infections	▪ Nausea ▪ Vomiting ▪ Liver problems ▪ Changes in how clearly one can think ▪ Insomnia ▪ Unwanted hair growth ▪ Confusion ▪	▪ Seizures ▪ Changes in vision ▪ Dizziness ▪ Red blood cell destruction

It is very important that you do not eat grapefruit or drink grapefruit juice while taking Tacrolimus. Grapefruit has an ingredient called bergamottin, which can affect some of the treatment drugs used in this study. Common soft drinks that have bergamottin are *Fresca*, *Squirt*, and *Sunny Delight*.

Cyclosporine A

Likely Side Effects (May happen in more than 20% of patients)	**Less Likely** (May happen in less than 20% of patients)	**Rare** (May happen in less than 2% of patients)
Kidney problems Loss of magnesium, calcium, and potassium High blood pressure	Liver problems Unwanted hair growth Growth of extra tissue on the gums Burning, tingling or numbness in the hands, arms, feet or legs	Seizures Changes in vision Formation of very small blood clots

<u>Risks and Toxicities Related to Transplant</u>
The following problems may occur as a result of stem cell transplantation. These risks may occur whether a transplant was done as part of the study or not:

Slow recovery of blood counts. The red blood cells, white blood cells, and platelets can be slow to recover after blood or marrow transplant. Until your blood counts recover, you will need blood and platelet transfusions, and will be at risk for bleeding and infections. To speed the recovery of the white cells as much as possible you may receive Filgrastim.

Graft failure. The stem cells (the "graft") may fail to grow inside your body. Past experience suggests that there can be up to a 10-15% chance of graft failure. If graft failure occurs, this may result in low blood counts for a long period of time. If your counts do not recover, you may need to receive a second transplant. Graft failure can be fatal.

Graft-Versus-Host Disease (GVHD). GVHD results from cells in the graft recognizing your body as foreign and attacking it. In most cases, GVHD can be successfully treated. Sometimes GVHD is severe or difficult to treat and may lead to death. You will be watched closely for this complication and given drugs to prevent and/or treat it.

Acute GVHD may produce skin rash, nausea, vomiting, diarrhea, abdominal pain, abnormalities of liver function, and an increased risk of infection. Chronic GVHD may produce skin rashes, hair loss, thickened dry skin, dry eyes, dry mouth, liver disease, weight loss, diarrhea, and an increased risk of infection. To confirm the diagnosis of acute or chronic GVHD, you may be asked to have a biopsy (a small sample of your tissue to look at under the microscope) of your skin, gut, or, rarely, your liver.

Other complications. Other complications may include:

a. Damage to the vital organs in your body. The transplant could cause problems in any body organ such as the heart, lungs, liver, gut, kidneys and bladder, or brain. The kidneys and the liver are most likely to be damaged. Some patients will experience serious lung problems from infections or the chemotherapy and radiation.

b. Serious infections. Full and complete recovery of your immune system may take many months. During this time, there is an increased risk of infections. You will be prescribed certain drugs to reduce the chance of those infections. However, these treatments do not always work. If you have an infection, you may have to stay in the hospital longer or be re-hospitalized after transplant. Although most infections can be successfully treated, some infections may result in death.

c. Relapse of disease or a new blood cancer. Your leukemia may come back even if the transplant is initially successful. In rare cases, a new blood cancer may develop from the donor cells. Cyclophosphamide can cause damage to blood cells, which may result in a blood cancer such as myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML). The blood cancer usually develops 2-10 years after treatment, or 6 years on average. The risk of developing a new blood cancer after allogeneic blood or marrow transplant is probably less than 2%. If cancer develops in your donor's blood cells, you may require additional treatment with chemotherapy or another blood or marrow transplant.

d. Lymphoproliferative Syndrome: Patients in Treatment Group A (CD34 Selected Peripheral Blood Stem Cell Graft) have an increased risk of developing post-transplant lymphoproliferative disorder (PTLD) or lymphoma caused by a virus called EBV. They can develop symptoms like fevers and enlarged lymph nodes. Your doctor may use scans and biopsies to confirm the diagnosis. Your blood will be monitored to check if you have signs of EBV in the blood. In many patients EBV can be treated at that stage before it ever progresses to lymphoma. EBV in the blood or EBV lymphoma often responds to treatment with rituximab, a drug commonly used in other lymphomas. PTLD can be fatal.

e. Risk to the unborn. The treatments in this study have <u>not</u> been proven to be safe at any stage of pregnancy. Therefore, if you are pregnant or nursing, you are not eligible for this study. Women who can become pregnant must use effective birth control while receiving chemotherapy, TBI, and drugs to prevent GVHD, and for 1 year after transplant. Effective birth control is defined as the following:

1. Refraining from all acts of vaginal sex (abstinence)
2. Consistent use of birth control pills

3. Injectable birth control methods (Depo-Provera, Norplant)
4. Tubal sterilization or male partner who has undergone a vasectomy
5. Placement of an IUD (intrauterine device)
6. Use of a diaphragm with contraceptive jelly and/or condoms with contraceptive foam every time you have sex.

Reproductive Risks

The drugs used in this research study may damage your reproductive organs, affect your ability to have children or possibly cause birth defects if you take them while you are pregnant. It is important that a woman is not pregnant or breast-feeding and does not become pregnant during the course of the study.

It is important that both women who can become pregnant and their male partners use birth control for 1 year after transplantation while on this study.

If you are a woman and can become pregnant, you will need to take a pregnancy test before you start the study. You should discuss ways to prevent pregnancy while you are in the study. Women who have gone through puberty may find that their menstrual cycle becomes irregular or stops permanently. This does not mean that you cannot become pregnant. You must still use an effective method of birth control during your transplant and continue until you are finished with your GVHD prevention treatment.

If you are a man, your body may not be able to produce sperm (become sterile). You should talk with your doctor about banking your sperm before having a transplant.

Please check with your doctor to understand more about these risks.

Unforeseen Risks

New risks might appear at any time during the study. These risks might be different from what is listed in this Consent Form. We will promptly tell you about new information that may affect your decision to take part in the study. We may learn new things that might make you want to stop being in the study. We will let you know if this happens and you can decide if you want to continue in the study.

Other Treatments or Medications

Some medicines react with each other, and it is important that you tell the study doctor or staff about any other drugs, treatments, or medicines you are taking. This includes non-prescription medications, vitamins and herbal treatments.

It is also important that you tell the study staff about any changes to these medications during your participation in the study.

For more information about risks and side effects, ask your study doctor.

7. Alternative Treatments

Participation in this study is optional. If you choose not to take part, you may still receive an allogeneic transplant to treat your disease. The treatment and evaluations you would receive could be very similar to what would receive if you join this study.

Your study doctor will talk with you about your options. If you decide not to participate in this study, your medical care will not be affected in any way.

Your other choices may include:

- Treatment with other drugs, radiation, or a combination of drugs and radiation without a transplant.
- An allogeneic blood or marrow transplant that is not part of the study, or another type of transplant
- Participation in another clinical trial, if available (check with your doctor)
- No treatment for your blood cancer at this time
- Comfort care

Every treatment option has benefits and risks. Talk with your doctor about your treatment choices before you decide if you will take part in this study.

8. Possible Benefits

Taking part in this study may or may not make your health better. The information from this study will help doctors learn more about medications used to prevent GVHD.

9. New Information Available During the Study

During this research study, the study doctors may learn about new information about the study drugs or the risks and benefits of the study. If this happens, they will tell you about the new information. The new information may mean that you can no longer participate in the study, or that you may not want to continue in the study.

If this happens, the study doctor will stop your participation in the study and will offer you all available care to suit your needs and medical conditions.

10. Privacy, Confidentiality and Use of Information

Your confidentiality is one of our main concerns. We will do our best to make sure that the personal information in your medical record is kept private. However, we cannot guarantee total privacy. All your medical and demographic (such as race and ethnicity, gender and household

income) information will be kept private and confidential. *(Name of Transplant Center)* and the organizations listed below will not disclose your participation by any means of communication to any person or organization, except by your written request, or permission, or unless required by federal, state or local laws, or regulatory agencies.

Individuals authorized by the organizations below will have access to your research and medical information. They may use this information for inspections or audits to study the outcomes of your treatment, or for required reporting to regulatory authorities (such as the FDA for serious adverse events). In agreeing to participate, you consent to such inspections and to the copying of parts of your records, if required by these organizations.

- The National Institutes of Health (NIH), which include the National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute (NCI)
- The Food and Drug Administration (FDA)
- The Blood and Marrow Transplant Clinical Trials Network Data and Coordinating Center (BMT CTN DCC), including the Center for International Blood and Marrow Transplant Research (CIBMTR), the National Marrow Donor Program (NMDP), and the EMMES Corporation.
- The BMT CTN Data and Safety Monitoring Board (DSMB)
- Miltenyi Biotec, makers of the device that removes cells that are associated with the development of GVHD (used in Treatment Group A)

We will not identify you by name in any publications or reports that come from these organizations or groups.

Information that does not include personally identifiable information about this clinical trial has been or will be submitted, at the appropriate and required time, to the government-operated clinical trial registry data bank, which contains registration, results, and other information about registered clinical trials.

This data bank can be accessed by you and the general public at www.ClinicalTrials.gov. Federal law requires clinical trial information for certain clinical trials to be submitted to the data bank.

Genetic Information Nondiscrimination Act:
A new federal law (2009), called the Genetic Information Nondiscrimination Act (GINA), generally makes it illegal for health insurance companies, group health plans, and employers of 15 or more persons to discriminate against you based on your genetic information. Health insurance companies and group health plans may not request your genetic information that we get from this research. This means that they must not use your genetic information when making decisions regarding insurability. Be aware that this new federal law will not protect you against genetic discrimination by companies that sell life insurance, disability insurance, or long-term care insurance.

11. Ending Your Participation

Being in this study is voluntary. You can choose to not be in this study, or leave this study at any time. If you choose not to take part or leave this study, your regular medical care will not be affected in any way. Tell your doctor if you are thinking about stopping or decide to stop. He or she will tell you how to stop safely.

The study doctor or the study sponsor may stop the study at any time, and we may ask you to leave the study. We may ask you to leave the study if you do not follow directions or if you suffer from side effects of the treatment. If we ask you to leave the study, the reasons will be discussed with you. Possible reasons to end your participation in this study include:

- You do not meet the study requirements.
- You need a medical treatment not allowed in this study.
- The study doctor decides that it would be harmful to you to stay in the study.
- You are having serious side effects.
- You become pregnant.
- You cannot keep appointments or take study drugs as directed.
- The study is stopped for any reason.

If you decide to leave this study after taking the study treatment, or are asked to leave by your doctor for medical reason, you will need to come back to the doctor's office for tests for your safety. Even if you leave the study, the information collected from your participation will be included in the study evaluation.

12. Physical Injury as a Result of Participation

It is important that you tell your doctor, _____ *[investigator's name(s)]* or study staff if you feel that you have been injured because of taking part in this study. You can tell the doctor in person or call him/her at _____ *[telephone number]*.

You will get medical treatment if you are injured as a result of taking part in this study. You and/or your health plan will be charged for this treatment. The study will not pay for medical treatment.

In case of injury resulting from this study, you do not lose any of your legal rights to seek payment by signing this form.

13. Compensation or Payment

You will not be paid for your participation in the research study. You will not get compensation or reimbursement for any extra expenses (travel, meals, etc.) you may have through your participation on this trial.

14. Costs and Reimbursements

Most of the visits for this research study are standard medical care for patients undergoing allogeneic transplants and will be billed to your insurance company. You and/or your health plan/insurance company will need to pay for some or all of the costs of standard treatment in this study.

You or your insurance will not be charged for optional blood samples for research on this study.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at http://cancer.gov/clinicaltrials/understanding/insurance-coverage. You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site. Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

15. Ethical Review

The ethical aspects of this research study have been reviewed and approved by [name of IRB].

16. For More Information

If you need more information about this study, or if you have problems while you are participating in this study, you can contact the study doctor or his/her staff. They can be reached at the telephone numbers listed here:

[*Insert name and contact details*]

17. Contact Someone about Your Rights

If you wish to speak to someone not directly involved in the study, or if you have any complaints about any aspect of the project, the way it is being conducted or any questions about your rights as a research participant, then you may contact:

[*Insert appropriate contact details*]

For questions about your rights while taking part in this study, call the _____*[name of center]* Institutional Review Board (a group of people who review the research to protect your rights) at _____ *(telephone number).*

18. *Optional* Blood Samples for Future Research

This section of the informed consent form is about optional future research studies. This research will be done using blood samples from patients who are taking part in the main study described above. You may give blood samples for these research studies if you want to. You can still be a part of the main study even if you say 'no' to giving blood samples for these research studies. You can say "yes" or "no" to giving these optional blood samples for research. Please mark your choice at the end of this section.

We would like to have five (5) blood samples for future research studies. If you agree, these samples will be drawn before you begin the conditioning regimen for your transplant (2 teaspoons or 6 mL), and at 4 different times after your transplant on Days 35, 100, 180, and 365 (20 teaspoons or 86 mL at each time point). **These samples will only be collected in patients who weigh more than 30.0 kg.** Usually the blood can be drawn from a vein in your arm at the same time as other blood collections.

The samples collected for future research purposes will be sent to the BMT CTN Repository. The samples will be labeled with unique codes that do not contain information that could identify you. A link to this code does exist. The link is stored at the Data and Coordinating Center for the Blood and Marrow Transplant Clinical Trials Network (BMT CTN DCC). The staff at the repository where your samples are being stored does not have a link to this code. Your research samples will continue to be stored at the BMT CTN Repository until they are used up for research.

The reason for collection of these samples is to perform future studies to better understand how your immune system recovers after the transplant. This will help understand why patients develop complications after transplant, including infections and graft-versus-host disease.

The research that may be done with your blood is not designed specifically to help you. It might help people who have cancer and other diseases in the future.

Reports about research done with your blood will not be given to you or your doctor. These reports will not be put in your health record. The research will not have an effect on your care.

Genome-Wide Association Studies:
DNA from your stored blood samples might be used in genome-wide association (GWA) studies for a future project either done or supported by the National Institutes of Health (NIH). Genome-wide association studies are a way for scientists to find genes that have a role in human disease or treatment. Each study can look at hundreds of thousands of genetic changes at the same time.

If your coded samples are used in such a study, the researcher is required to add your test results and sample information into a shared, public research database. This public database is called the NIH Genotype and Phenotype Database and it is managed by the National Center for Biotechnology Information (NCBI). The NCBI will never have any information that would

identify you, or link you to your information or research samples, although the results of genetic studies could theoretically include identifying information about you.

Things to Think About:
The choice to let us have blood samples for future research is up to you. No matter what you decide to do, it will not affect your care.

If you decide now that your blood can be kept for future research, you can change your mind at any time. Just contact your study doctor and let him or her know that you do not want us to use your blood sample. Then any blood that remains will no longer be used for research.

In the future, people who do research on these blood samples may need to know more about your health. While the study doctor or others involved in running this study may give the researchers reports about your health, it will not give them your name, address, phone number, or any other information that will let the researchers know who you are.

Your blood will be used only for research and will not be sold. The research done with your blood may help to develop new products in the future.

Benefits:
The benefits of research using blood include learning more about how your body's immune system recovers after a transplant, as well as why certain complications like graft-versus-host disease or infections develop.

Risks:
There is a small risk of an infection or fainting from the blood draw.

The greatest risk to you is the release of information from your health records. We will do our best to make sure that your personal information will be kept private. The chance that this information will be given to someone else is very small.

Making Your Choice:
Please read each sentence below and think about your choice. After reading each sentence, please indicate your choice below. If you have any questions, please talk to your doctor or nurse, or call our research review board at _____.

No matter what you decide to do, it will not affect your care.

Statement of Consent

The purpose of storing blood samples, the procedures involved, and the risks and benefits have been explained to me. I have asked all the questions I have at this time and I have been told whom to contact if I have more questions. I have been told that I will be given a signed copy of this consent form to keep.

I understand that I do not have to allow the use of my blood for research. If I decide to not let you store research samples now or in the future, it will not affect my medical care in any way.

I voluntarily agree that blood samples may be collected and that my blood and related information can be stored indefinitely by the BMT CTN Repository for research on how my body's immune system recovers after the transplant.

- ❑ I <u>do</u> agree to give optional blood samples for future research.
- ❑ I <u>do not</u> agree to give optional blood samples for future research.

______________________________________ ___________
Signature Date

♦ ♦

Health Insurance Portability and Accountability Act 1 (HIPAA[3]) Authorization to use and disclose individual health information for research purposes

A. **Purpose**:
As a research participant, I authorize the Principal Investigators and the researcher's staff to use and disclose my individual health information for the purpose of conducting the research study:

A Randomized, Multi-Center, Phase III Trial of Calcineurin Inhibitor-Free Interventions for Prevention of Graft-versus-Host Disease

B. **Individual Health Information to be Used or Disclosed**:
My individual health information that may be used or disclosed to do this research includes:

- Demographic information (for example: date of birth, sex, weight)
- Medical history (for example: diagnosis, complications with prior treatment)
- Findings from physical exams
- Laboratory test results obtained at the time of work up and after transplant (for example: blood tests, biopsy results)

C. **Parties Who May Disclose My Individual Health Information**:
The researcher and the researcher's staff may collect my individual health information from:
[*List hospitals, clinics or providers from which health care information can be requested*]

D. **Parties Who May Receive or Use My Individual Health Information**:
The individual health information disclosed by parties listed in item c and information disclosed by me during the course of the research may be received and used by the following parties:

<u>Study Sponsors</u>

- National Heart, Lung, and Blood Institute (NHLBI) and the National Cancer Institute (NCI), both of the National Institutes of Health (NIH)
- Blood and Marrow Transplant Clinical Trials Network Data and Coordinating Center (BMT CTN DCC) , including the Center for International Blood and Marrow Transplant Research (CIBMTR), the National Marrow Donor Program (NMDP), and the EMMES Corporation.
- BMT CTN 1301 Co-Principal Investigators: Dr. Leo Luznik, Dr. Marcelo Pasquini, and Dr. Miguel Angel Perales.

<u>Other Organizations</u>

[3] HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information

- U.S. government agencies that are responsible for overseeing research such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP)
- U.S. government agencies that are responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments
- Miltenyi Biotec, makers of the device that removes cells that are associated with the development of GVHD (used in Treatment Group A)

E. **Right to Refuse to Sign this Authorization**:
I do not have to sign this Authorization. If I decide not to sign the Authorization, I will not be allowed to participate in this study or receive any treatment related to research that is provided through the study.

My decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.

F. **Right to Revoke**:
I can change my mind and withdraw this authorization at any time by sending a written notice to the Principal Investigator to inform the researcher of my decision.

If I withdraw this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about me will be collected by or disclosed to the researcher for this study.

G. **Potential for Re-disclosure**:
My individual health information disclosed under this authorization may be subject to re-disclosure outside the research study and no longer protected.

Examples include potential disclosures for law enforcement purposes, mandated reporting or abuse or neglect, judicial proceedings, health oversight activities and public health measures.

H. **This authorization does not have an expiration date.**

I have read and understood this Consent Form. The nature and purpose of the research study has been explained to me.

- I have had the chance to ask questions, and understand the answers I have been given. I understand that I may ask questions at any time during the study.
- I freely agree to be a participant in the study.
- I understand that I may not directly benefit from taking part in the study.
- I understand that, while information gained during the study may be published, I will not be identified and my personal results will stay confidential.
- I have had the chance to discuss my participation in this research study with a family member or friend.
- I understand that I can leave this study at any time, and doing so will not affect my current care or prevent me from receiving future treatment.
- I understand that I will be given a copy of this signed consent form.

_____ _____
Participant Name Date

_____
Signature Date

If you are not the subject, please print your name_____
and indicate one of the following:

_____	The subject's parent	_____	The subject's guardian
_____	A surrogate	_____	A durable power of attorney
_____	A proxy	_____	Other, please explain:

_____ _____
Legally Authorized Representative Signature Date

I certify that I have provided a verbal explanation of the details of the research study, including the procedures and risks. I believe the participant has understood the information provided.

_____

Name of Counseling Physician Date

_____

Signature of Counseling Physician Date

CONSENT AND ASSENT INSTRUCTIONS

CONSENT: Subjects 18 years and older must sign on the Subject Signature line below. For subjects under 8 years old, consent is provided by the Legally Authorized Representative.

ASSENT: Is required for subjects ages 7 to 17, using the Assent Section on the following page.

I have been informed about this study's purpose, procedures, possible benefits and risks. I have been given the chance to ask questions. My questions have all been answered satisfactorily. I understand that I can ask other questions at any time.

I voluntarily agree to take part, or to allow my child to take part, in this study.

By signing this consent form, I have not given up any of the legal rights that I (my child) otherwise would have as a subject in a research study.

_____ _____

Subject‘s Signature Date

Pediatric Assent to Participate in Research

For Children Ages 7 to 17 years old

Study Title: A Randomized, Multi-Center, Phase III Trial of Calcineurin Inhibitor-Free Interventions for Prevention of Graft-versus Host-Disease

Protocol: BMT CTN # 1301

- **Why am I here?**

We are inviting you to join our study because you will receive a stem cell transplant to treat your disease. A transplant uses blood-making cells from another person (donor) to replace your cells that are not healthy. A donor is the name for a person who gives some of their blood-making cells for a transplant.

- **Why are you doing the study?**

We are comparing three different ways (types) to do a transplant to learn if any of them are better. Sometimes the donor cells cause a problem called graft versus host disease (GVHD). GVHD happens when the donor cells attack your body. One way to avoid this problem is to give medications, other ways include removing the donor cells that can attack your body before injecting them to you or killing them after they are injecting in you. These are the three types of transplant that are being compared in this study.

- **What will happen to me?**

If you participate in this study you will receive one of the transplant types which will be chosen by chance, like a flip of a coin.

Before your transplant, your will have check-ups with your doctors. Then, you will get a small tube put in your chest in the operating room (you will be asleep for this). The small tube makes it easier for you to get your medications. It will also make it easier and less painful for drawing blood for tests.

You will receive medicines that will help the cells from your donor grow in your body. These medicines might make you feel sick. You might throw up, lose your hair, or get sores in your mouth.

After you are done taking the medicines, you will get cells from your donor. This is your transplant. Your donor can be your sister or brother (related to you) or someone you don’t know (unrelated to you). You new cells will come from your donor’s bone marrow. The cells will make new and healthy cells in your body.

You will stay in the hospital for several days before your transplant and for about 4 weeks after your transplant. After you go home, you will need to go back to see your doctor often.

It is possible that your disease will come back after the transplant. If this happens, your doctor will find another way to treat you.

- **Will it Hurt?**

For your transplant, we will put a small tube in your chest. It might hurt a little and you might bleed a little. You will need blood drawings sometimes from your arm, which also might hurt a little. Your doctor and nurses will make sure you feel as little pain as possible.

- **Will the Study help me?**

We don't know if the study will help you or not.

- **What if I have questions?**

You can ask any question that you have about the study. If you forget to ask a question and think of it later, you can call me [*insert office number*]. You can also ask your question the next time you see me.

- **Do I have to be in the study?**

Writing your name on this page means that you agree to be in the study and know what will happen to you. If you decide to quit the study, all you have to do is tell your doctor.

You and your parent or guardian will get a copy of this form after you sign it.

_____ _____ _____
Signature of Child Date Age (years)

_____
Print Name of Child

Certification of Counseling Healthcare Professional: I certify that the nature and purpose, the potential benefits, and possible risks associated with participation in this study have been explained to the above individual and that any questions about this information have been answered.

_____ _____
Counseling Healthcare Professional Date

APPENDIX B-2

RELATED DONOR INFORMED CONSENT

Related Donor Informed Consent to Participate in Research

This is an informed consent document for a research study that your family member is participating in. This document will inform you about the details of this study, which will involve manipulation of your donated cells before they are given to your family member.

Your family member has a blood cancer and will be treated with a stem cell transplant under a research study. The goal of this study is to compare 2 different combinations of treatment plans to a standard transplant procedure. The objective is to see whether one or both of these treatment plans are better at reducing the occurrence of chronic graft versus host disease (GVHD), a life-threatening complication of transplant. The treatment plan that your family member was randomized to requires manipulation of your cells through a device that removes certain types of cells that can cause this complication.

This informed consent document will explain important information about the study. There is no additional requirement from you beyond the procedure of stem cell collection to which you have already agreed. The cells you donate will be manipulated using a cell selection system that is part of the research study. Therefore, you need to be informed about this process and consent that your cells can be manipulated according to the procedures in the study. It is important to know that:

- You will not be paid to be in this study.
- You, your medical insurance company, or the patient's medical insurance company will pay for all medical bills for your treatment and the cell manipulation procedure.

Before you decide on consenting and signing this document, please read the information below. Feel free to ask questions to understand your rights. The consent process is voluntary and will not interfere with your donation and the recipient's transplant.

1. Title of Research Study

A Randomized, Multi-Center, Phase III Trial of Calcineurin Inhibitor-Free Interventions for Prevention of Graft-versus-Host Disease

2. Principal Investigator Contact Information at your Institution

Name/Title/Phone number/

3. Contact information for emergencies after hours or on weekends or holidays:

Name/Phone number/

4. Sponsors and Source of Funding or Other Material Support

The National Institutes of Health (NIH), through the Blood and Marrow Transplant Clinical Trials Network (BMT CTN), are providing staff support and money for this research study. The BMT CTN and the NIH will make decisions about how to manage the study. Miltenyi Biotec, a company that produces the cell selection system used to process the stem cells before giving them to patients, is also a contributor to this study and they are providing supplies and money. This research study is also registered with the US Food and Drug Administration (FDA) which is overseeing the investigational device that is part of the cell selection system that will be used to remove T cells from your stem cell donation, called stem cell manipulation, prior to transplantation.

5. What will be different for you as a donor of peripheral blood stem cells if you choose to participate in this study?

Participation in this study by signing this document will not change any aspect of the stem cell donation that you have agreed to already. By signing this document, you acknowledge that your family member is participating in a research study and that you consent that the cells you donate can be manipulated in an investigational device.

6. What is the purpose of this study?

The purpose of this study is to compare two different combinations of treatment plans to a standard transplant procedure in order to see whether one or both of them are better at reducing the occurrence and severity of chronic GVHD. The research portion of this study involves manipulation of your stem cell product by removing T-cells, which cause chronic GVHD.

7. What will be done if you take part in this research study?

By signing this document, you consent that, after your donation, your cells will be manipulated by removing certain cells that can cause chronic GVHD in the recipient. The manipulation is performed by the investigational device. The way that you donate stem cells will not be changed with your participation in the study.

T-Cell Depletion (CD34+ Selection)

The blood cells collected from you as part of the donation process will have large numbers of T cells, along with other cells, including your blood stem cells. The process of removing these T cells is called T-cell depletion and there are several ways that this can be done. In this study, they are being removed through a process called negative selection. The device used to do this is called CliniMACS and is produced by Miltenyi Biotec. The procedure involves labeling the stem cells, also called CD34+ cells, with an antibody attached to a magnetic substance. The CliniMACS tubing set has columns that will bind only the CD34+ cells allowing all the other cells, including the T cells, to pass through. After the selection is done, the stem cell product will have mainly CD34+ cells, or stem cells, and will be depleted of T cells. The CliniMACS device has been extensively used in transplantation and has been proven to be safe. However, it remains an investigational device. This means it is not yet approved by the FDA for routine use. The FDA has granted approval for the use of this device and the CD34 reagent in this study and the study investigators are required to tell the FDA all information related to what happens with study participants. The CliniMACS CD34 Reagent System was approved as a

humanitarian device and authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this use has not been demonstrated.

8. Will you provide blood samples for research?

You will not be asked to provide extra blood samples for this research study.

9. What are the possible discomforts and risks?

T-cell Depletion: The process of manipulation occurs after your donation is completed. The amount of cells requested for collection from you is not larger than what is routinely requested for a transplant. However, the amount of cells donated needs to be above a certain number and some donors may need an extra day of donation in order to achieve this number of cells. Results from earlier studies using the same cell manipulation procedures found that 36% of the time, only 1 collection was needed; 45% of the time, 2 collections were needed; and 7% of the time, a third collection was needed.

Breach of Confidentiality: Medical records are considered confidential. These records are kept in a secured area accessible to people involved in the conduct of the study. You will not be identified by name in any publication or presentation of the results of this study. All data entered into a computer will be coded. No data that may be linked to you will be entered on any network computer that could allow access to confidential information. The master list will be stored off-line and available only to the principal investigator and his or her designee(s). Although we will make every effort possible to maintain confidentiality, there is however, a slight risk of loss of confidentiality.

10. As with any treatment, there may be yet unknown and/or unexpected side effects from donating peripheral blood stem cells.

Donating blood stem cells is routinely done and is not considered research. Unanticipated side effects may occur that have not been previously reported. If you have any unusual symptoms, you should report them immediately to your doctor.

In an attempt to avoid side effects, your doctor will examine you and obtain laboratory tests (blood tests, chest x-ray, etc.) to determine the effects of the donation and alter the drug doses if necessary.

11. What other alternatives are available if you do not want to be in this study?

Your participation is voluntary and you may choose not to participate in this research study or withdraw your consent at any time. Your choice will not at any time affect the commitment of your health care providers to provide care to you or to your family member. There will be no penalty or loss of benefits to which you or your family member are otherwise entitled. Alternatives to participating in this research include donating your blood stem cells to your family member for a transplant that is not part of this research study.

12. What are the possible benefits to you?

You will not benefit directly from participating in this research. You may receive indirect benefit from knowing that you may be helping your family member or other donors and patients in the future.

13. What are the possible benefits to others?

You may be helping other patients get better treatment in the future.

14. If you choose to take part in this study, will it cost you anything?

Normally the insurance company of the patient covers the medical expenses associated with collecting your blood stem cells and the T-cell depletion procedure. This will be reviewed with the patient's insurance company prior to collecting your stem cells. Neither you (the donor) nor your insurance company will be charged for the T-cell depletion of the peripheral blood stem cell graft.

15. Will you be paid for taking part in this research study?

No.

16. How can you withdraw from this research study?

If you change your mind after you have provided consent, you can still decline participation in the study prior to the stem cell manipulation. Please contact the person who discussed this document with you and request to be withdrawn from the study. The stem cell manipulation will occur within 24 to 36 hours from your donation.

17. How will your privacy and the confidentiality of your research records be protected?

The centers and doctors in charge of this study will keep your personal information as private as possible. They will do their best to see that it is shared only when required by state or federal law or the terms of this consent. The research study that your family member is participating in will not be collecting any information about you as the donor.

Information that does not include personally identifiable information about this clinical trial has been or will be submitted, at the appropriate and required time, to the government-operated clinical trial registry data bank, which contains registration, results, and other information about registered clinical trials. This data bank can be accessed by you and the general public at www.ClinicalTrials.gov. Federal law requires clinical trial information for certain clinical trials to be submitted to the data bank.

18. How will the researcher(s) benefit from you being in this study?

The researchers have no money invested in this study. But, in general, presenting research results helps the career of a scientist. Therefore, the Principal Investigator may benefit if the results of this study are presented at scientific meetings or in the scientific press. In addition, the Principal Investigator is being paid a small amount to cover the costs of the study.

19. HIPAA[1] authorization to use and disclose individual health information for research purposes

a. Purpose: As a research participant, I authorize the Principal Investigator and the researcher's staff to use and disclose my individual health information for the purpose of conducting the research study entitled *A Randomized, Multi-Center, Phase III Trial of Calcineurin Inhibitor-Free Interventions for Prevention of Graft-versus-Host Disease.*

b. Individual Health Information to be Used or Disclosed: My individual health information that may be used or disclosed to conduct this research includes: demographic information (e.g., age, date of birth, sex, weight), medical history (e.g., diagnosis, complications with prior treatment) and physical examination findings.

c. Parties Who May Disclose My Individual Health Information: The researcher and the researcher's staff may obtain my individual health information from (*list hospitals, clinics or providers from which health care information can be requested*).

_____

_____

_____

d. Parties Who May Receive or Use My Individual Health Information: The individual health information disclosed by parties listed in item "c." above and information disclosed by me during the course of the research may be received and used by the following parties:

- Principal Investigator and the researcher's staff
- Dr. Marcelo Pasquini, Study Chairperson at Medical College of Wisconsin
- Dr. Miguel Perales, Study Chairperson at Memorial Sloan-Kettering Cancer Center
- Dr. Leo Luznik, Study Chairperson at Johns Hopkins University
- National Heart, Lung and Blood Institute (NHLBI) and National Cancer Institute (NCI), both of the National Institutes of Health (NIH), study sponsors
- Blood and Marrow Transplant Clinical Trials Network Data and Coordinating Center (BMT CTN DCC), including the Center for International Blood and Marrow Transplant Research (CIBMTR), the National Marrow Donor Program (NMDP), and the EMMES Corporation
- The BMT CTN Data and Safety Monitoring Board (DSMB)
- U.S. government agencies that are responsible for overseeing research such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP)

[1] HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information.

- U.S. government agencies that are responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments
- Miltenyi Biotec, makers of the device that removes cells that are associated with the development of GHVD (used in the treatment group that your family member was randomized to)

e. Right to Refuse to Sign this Authorization: I do not have to sign this Authorization. If I decide not to sign the Authorization, I will not be allowed to participate in this study or receive any research-related treatment that is provided through the study. However, my decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.

f. Right to Revoke: I can change my mind and withdraw this authorization at any time by sending a written notice to the Principal Investigator to inform the researcher of my decision. If I withdraw this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about me will be collected by or disclosed to the researcher for this study.

g. Potential for Re-disclosure: My individual health information disclosed under this authorization may be subject to re-disclosure outside the research study and no longer protected. Examples include potential disclosures for law enforcement purposes, mandated reporting or abuse or neglect, judicial proceedings, health oversight activities and public health measures.

h. This authorization does not have an expiration date.

20. Donor's Consent

I have been informed of this study's purpose, procedures, possible benefits and risks. I have been given a chance to ask questions and have had them answered to my satisfaction. I understand that I can ask more questions at any time.

I voluntarily agree to participate in this study.

By signing this consent form, I have not given up any of the legal rights, which I otherwise would have as a subject in a research study.

_____ _____
Signature of Donor Date

_____
Print Name of Donor

Certification of Counseling Healthcare Professional

I certify that the nature and purpose, the potential benefits, and possible risks associated with participation in this study have been explained to the above individual and that any questions about this information have been answered.

_____ _____
Signature of Counseling Healthcare Professional Date

_____
Print Name of Counseling Healthcare Professional

Related Donor Informed Assent to Participate in Research

This is a form for a research study. This form is to help you decide if you want to participate in this study.

Purpose of the Research Study
Your brother or sister has blood cancer and is being treated with a transplant of peripheral blood stem cells from a matched family member donor in a research study.

The goal of this study is to compare 2 different treatment plans to a standard transplant procedure. The objective is to see whether one or both of these treatment plans are better at reducing the rate of a serious complication called chronic graft versus host disease (GVHD). The treatment plan that your brother or sister was assigned to requires putting your donated cells through a device that removes certain types of cells that cause this complication before the cells are transplanted to your brother or sister.

You are being asked to be in the study because you are a match for your brother or sister and can donate peripheral blood stem cells to them. Your doctor or another person on the study team will explain to you what you must do if you are going to donate peripheral blood stem cells for your brother or sister. The team will also follow you closely to see if you are having any side effects while donating peripheral blood stem cells on the study.

If you have any questions, ask your doctors and make sure you understand their answers. Your parents (or a guardian) are also asked for their permission for you to join this treatment study.

I agree to donate blood stem cells in this study.

_____ _____
Signature of Donor Date

_____
Print Name of Donor

_____ _____
Signature of Doctor Date

_____
Print Name of Doctor

APPENDIX C

LABORATORY PROCEDURES

APPENDIX C

LABORATORY PROCEDURES

OPTIONAL FUTURE RESEARCH: PROPOSED DETAILED IMMUNOLOGIC STUDIES

Patients consenting to the *optional* future research will have samples collected for undefined future research, as well as immune reconstitution and proposed detailed immunological studies. Samples will be collected at Baseline (prior to the initiation of the conditioning regimen) and post-transplant at Days 35, 100, 180 and 365. These samples will be shipped for cryopreservation to the BMT CTN Repository and processed for later analysis. The BMT CTN Repository will process samples according to standard operating procedures (SOPs). Table 1 describes allocation of specimens for immunologic analysis.

Patients who weigh more than 30.0 kg are eligible to provide optional research samples and will have 6 mL of whole blood collected at Baseline and 80 mL of whole blood collected at each post-transplant time point.

Proposed Experimental Approach

Multi-parameter flow cytometric analysis of effector and regulatory T-cell subsets: The flow cytometric examination of effector and regulatory T cells will be conducted on blood samples on cryopreserved samples. Based on established SOPs by the NMDP repository each sample will be aliquoted in multiple vials with a cell viability upon freeze thaw of >95%. This "central" sample processing is expected to provide maximal viability and functionality of the cells. Shipment of specimens between NMDP repository and institutions will occur in batches and will start in year 2 of patient accrual. The proposed studies are designed to assess the impact of GVHD prophylaxis strategy on reconstitution of lymphocyte subsets, particularly balance of alloreactive effector and regulatory T cells and the relationship between GVHD prophylaxis and thymus function. Lymphocyte subsets will be identified using the following panels: CD4 subsets: naïve, effector, effector memory and central memory subpopulations defined by CD4 CD45RA CCR7 CD38 HLA-DR markers; recent thymic emigrants identified by the CD4+ CD45RA+ CD31+ CD95 dim phenotype.[75, 76, 77, 78] CD8 subsets: naïve, effector, effector memory and central memory subpopulations defined by the CD8 CD45RA CCR7 CD38 HLA-DR markers;[75, 79, 80] recent thymic emigrant identified by the CD8+CD45RA CD31+ CD95dim phenotype. B cell subsets: memory versus naïve B cell populations will be delineated by their expression of CD27, IgM and IgD.[81] NK cell subsets: (CD56 bright, CD16 neg and CD56 moderate, CD16+). The analysis of regulatory T cells will be performed using multi-color flow cytometry with a pre-determined panel of monoclonal antibodies (mAbs) including but not limited to those specific for CD3, CD4, CD25 (IL-2Rα), CD31 (PECAM1), CD45RA, CD127 (IL-7R), Ki-67 and intracellular Foxp3. This multi-color approach would allow phenotypic separation of human $CD4^{+}Foxp3^{+}$ T cells into three distinct subpopulations as well as assessment of their proliferative status (percentage of Ki-67 positive cells).[82, 83] In addition, we will combine these stains with Bcl-2, CD95, and Annexin V stains to assess the apoptotic susceptibility of donor Tregs. In all analyses the frequency and total number of cells will be determined.

Table 1: Allocation of Specimens for Immunologic Analysis and Future Research

***Optional* Research Samples**							
Subjects	**Research Sample Type**	**Time Points**	**Sample Quantity**	**Stored Material**	**Sample Processing & Storage Site**	**Aliquots Stored**	**Proposed Studies**
Patients who weigh > 30.0 kg.	Peripheral Blood	Pre-Transplant prior to the initiation of conditioning regimen	6 mL EDTA	Whole Blood	BMT CTN Repository	Maximum 6 aliquots 1.0 mL whole blood aliquots stored at -80° C	Undefined Future Research (Genomic DNA Isolation)
		Post-Transplant Days 35, 100, 180, and 365	80 mL Heparin	Viable PBMCs	BMT CTN Repository	Maximum 12 1.0 mL aliquots containing ~5.0-10.0 x 10^6 PBMC; controlled-rate frozen and stored in LN	Advanced Immune Reconstitution **(MSKCC)**
							Tregs assays/ multi-color flow cytometry **(Johns Hopkins)**
			6 mL Clot	Serum	BMT CTN Repository	Maximum 6 0.5 mL plasma aliquots stored at -80° C	Undefined Future Research (Proteomic)

APPENDIX D

ESTIMATED CREATININE CLEARANCE FORMULAS

APPENDIX D

ESTIMATED CREATININE CLEARANCE FORMULAS

Cockcroft-Gault (*for patients > 12 years*)

$$\text{Estimated CrCl} = \frac{(140 - \text{age}) \times \text{Mass (in kilograms)} \times [0.85 \text{ if Female}]}{72 \times \text{Serum Creatinine (in mg/dL)}}$$

Updated Schwartz (*for patients ≥ 1 year to 12 years*)

$$\text{Estimated GFR} = \frac{k \times \text{Height (in centimeters)}}{\text{Serum Creatinine (in mg/dL)}}$$

Where *k* is a constant that depends on muscle mass, which varies with a child's age:

- In first year of life, for pre-term babies *k*=0.33and for full-term infants *k*=0.45
- For infants and children of age 1 to 12 years, *k*=0.55.

APPENDIX E

KARNOFSKY AND LANSKY PERFORMANCE STATUS SCALES

APPENDIX E

KARNOFSKY AND LANSKY PERFORMANCE STATUS SCALES

Karnofsky for patients ≥ 16 years

Index	Specific Criteria	General
100	Normal, no complaints, no evidence of disease.	Able to carry on normal activity; no special care needed.
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 for self, unable to carry on normal activity or to do work.	Unable to work, able to live at home and care for most personal needs, varying amount of assistance needed.
60	Requires occasional assistance from others but able to care for most needs.	
50	Requires considerable assistance from others and frequent medical care	
40	Disabled, requires special care and assistance.	Unable to care for self, requires institutional or hospital care or equivalent, disease may be rapidly progressing.
30	Severely disabled, hospitalization indicated, but death not imminent.	
20	Very sick, hospitalization necessary, active supportive treatment necessary.	
10	Moribund	
0	Dead	

Lansky for patients < 16 years

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

0	Dead

APPENDIX F

HCT-SPECIFIC COMORBIDITY INDEX SCORE

APPENDIX F

HCT-SPECIFIC COMORBIDITY INDEX SCORE

Comorbidities	Definition	Score
Migraine/headache		**0**
Osteoporosis		**0**
Osteoarthritis		**0**
Hypertension		**0**
Gastrointestinal	Including inflammatory bowel disease	**0**
Mild pulmonary	DLCo and/or FEV_1 >80% or Dyspnea on moderate activity	**0**
Mild renal	Serum creatinine 1.2-2 mg/dl	**0**
Endocrine		**0**
Bleeding		**0**
Coagulopathy	Deep venous thrombosis or pulmonary embolism	**0**
Asthma		**0**
Arrhythmia		**1**
Myocardial	Coronary artery disease, congestive HF, history of medically documented MI, EF≤50%	**1**
Mild hepatic	Chronic hepatitis, Bilirubin >ULN- 1.5 X ULN, or AST/ALT >ULN-2.5XULN	**1**
Cerebro-vascular accident	History of transient ischemic attack or cerebro-vascular accident	**1**
Morbid obesity		**1**
Diabetes	Requiring treatment	**1**
Depression/anxiety		**1**
Infection	Requiring continuation of treatment after Day 0	**1**
Rheumatologic	SLE, RA, polymyositis, mixed CTD, polymyalgia rheumatica	**2**
Moderate pulmonary	DLCo and/or FEV_1 66-80% or Dyspnea on slight activity	**2**
Peptic ulcer	Patients who have required treatment	**2**
Moderate-severe renal	Serum creatinine >2 mg/dl, on dialysis, or prior renal transplantation	**2**
Valvular heart disease	Except mitral valve prolapse	**3**
Prior solid tumor	Requiring treatment with chemotherapy	**3**
Moderate-severe hepatic	Liver cirrhosis, Bilirubin >1.5 X ULN, or AST/ALT >2.5XULN	**3**
Severe pulmonary	DLCo and/or FEV_1 ≤65% or Dyspnea at rest or requiring oxygen	**3**

Total score is the sum of all comorbidities present pre-transplant.

APPENDIX G

DERIVATION OF A SEQUENTIAL TEST STATISTIC FOR CENSORED EXPONENTIAL DATA

APPENDIX G

DERIVATION OF A SEQUENTIAL TEST STATISTIC FOR CENSORED EXPONENTIAL DATA

Background – The Sequential Probability Ratio Test

Let $f(.,\theta)$ be the density function for random variable X. According to Neyman and Pearson, the most powerful test of $H_0 : \theta = \theta_o$ versus $H_1 : \theta = \theta_1$ decides in favor of H_1 or H_0 if $L_n > c_\alpha$ or $L_n < c_\alpha$, respectively, where $L_n = \prod_i^n f(x_i;\theta_1) / f(x_i;\theta_0)$ is the likelihood ratio, and c_α is determined to have the size α. When the sample size is not fixed in advance, further improvement is possible by using Wald's Sequential Probability Ratio Test (SPRT). The SPRT continues to sample as long as $B < L_n < A$ for some constant $B < 1 < A$, stops sampling and decides in favor of H_1 as soon as $L_n > A$, and stops sampling and decides in favor of H_0 as soon as $L_n < B$.

The usual measures of performance of such a procedure are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$, and of accepting H_0 when $\theta = \theta_1$, respectively, and the expected sample size $E(N \mid \theta_j) \equiv E_j(N)$. Wald and Wolfowitz showed that among all tests, sequential or not, for which $\Pr_0(\text{reject } H_0) \le \alpha$ and $\Pr_1(\text{reject } H_0) \le \beta$, and for which $E_j(N)$ are finite, j=0,1, the SPRT with error probabilities α and β minimizes $E_0(N)$ and $E_1(N)$. If, in addition, the $x1, x2,...$ are independent and identically distributed (i.i.d.) with density function $f(x,\theta)$, with monotone likelihood ratio in $\tau(x)$, then any SPRT for testing θ_0 against $\theta_1 (> \theta_0)$ has non-decreasing power function.

For the SPRT with error probabilities α and β, the SPRT boundaries are given approximately by $A = (1-\beta)/\alpha$ and $B = \beta/(1-\alpha)$. The operating characteristics of the SPRT are given by $O(\theta,\alpha,\beta,\theta_0,\theta_1) = (A^{h(\theta)} - 1)/(A^{h(\theta)} - B^{h(\theta)})$ where $h(\theta)$ is the non-trivial solution to the equation $\int (f(x;\theta_1)/f(x,\theta_2))^{h(\theta)} f(x;\theta)dx = 1$.

The formula $E(N;\theta) = [[(1-O(\theta)]\log A + O(\theta)\log B]/E(z;\theta)$ provides the average sample number for an arbitrary θ. The sample size distribution is very highly skewed, $Var(N) \approx [E(N)]^2$. Thus we will consider a truncated test with maximum sample size of N_0 and simulate to obtain the operating characteristics of the test.

Derivation of the SPRT for Censored Exponential Survival Times

Suppose that we wish to construct a sequential test for the composite null hypothesis that the rate of overall mortality at an early time point t is less than or equal to p_0 versus the alternative hypothesis that it is greater than or equal to p_0. Let us assume that the survival times, $T_1, T_2, ..., T_n$, are i.i.d. with exponential density function $f(T,\theta) = \theta e^{-\theta T}$. Although an exponential model may not fit well for overall mortality, it usually provides a reasonable model over a short time frame for modeling toxicity, so in all discussion below we assume that exponential survival times are censored at time point t. In the exponential parameterization, a t-day survival rate of p_0 translates into a mean survival of μ_0=-t/ln(1-p_0) (rate parameter θ_0 = -ln(1-p_0)/t). .

The SPRT is derived with reference to a simple null and alternative hypothesis for the rate parameter, in this case, $H_0 : \theta = \theta_o$ versus $H_1 : \theta = \theta_1$. The log-likelihood ratio for the exponential in the presence of censoring is $\log \prod_i^n f(x_i;\theta_1) - \log \prod_i^n f(x_i,\theta_0) = d(\log(\theta_1) - \log(\theta_0)) - (\theta_1 - \theta_0)\sum_i^n T_i$, where d is the number of events. The SPRT can be represented graphically when plotting the number of deaths (d) on the y axis against the total time on study $\sum_i^n T_i$ on the x axis. The continuation region in terms of d is bounded by two parallel lines given by

$$\left[\frac{\log(B)}{(\log\theta_1 - \log\theta_0)}\right] + \left[\frac{(\theta_1 - \theta_0)}{(\log\theta_1 - \log\theta_0)}\right]\sum_i^n T_i < d < \left[\frac{\log(A)}{(\log\theta_1 - \log\theta_0)}\right] + \left[\frac{(\theta_1 - \theta_0)}{(\log\theta_1 - \log\theta_0)}\right]\sum_i^n T_i$$

with common slope $(\theta_1 - \theta_0)/(\log\theta_1 - \log\theta_0)$, and intercepts $\log A/(\ln\theta_1 - \ln\theta_0)$ and $\log B/(\ln\theta_1 - \ln\theta_0)$, for the upper and lower bounds, respectively. For monitoring purposes, at an interim analysis calendar time point s, suppose that d(s) events have occurred and that the total time on study is $\sum_i^n T_i(s)$.The cumulative number of events d(s) is plotted on the y axis against the total time on study, $\sum_i^n T_i(s)$. When this graph crosses the upper boundary, the null hypothesis is rejected. In practice, monitoring will be scheduled monthly after the start of enrollment to the study.

A truncated version of the SPRT can be obtained by specifying a maximum sample size. We truncate the SPRT by declaring that if the test has failed to terminate after the maximum sample size, that the null hypothesis will be accepted. Since the probability that the untruncated SPRT would reject the null at the maximum sample size is negligible, it makes little difference how the final boundary value is selected, and this rule is chosen for simplicity. The operating characteristics of this proposed truncated SPRT for censored exponential data can be estimated by simulation.

APPENDIX H

REFERENCES

APPENDIX H

REFERENCES

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