Myeloablative Unrelated Donor Cord Blood Transplantation with T -Cell Depleted Haplo-identical Peripheral Blood Stem Cells for Patients with High Ri sk Hematological Malignancies

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOC ɔL

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Please Note: A Consenting Professional must have completed the mandatory Human Subjects Education and Certification Program.

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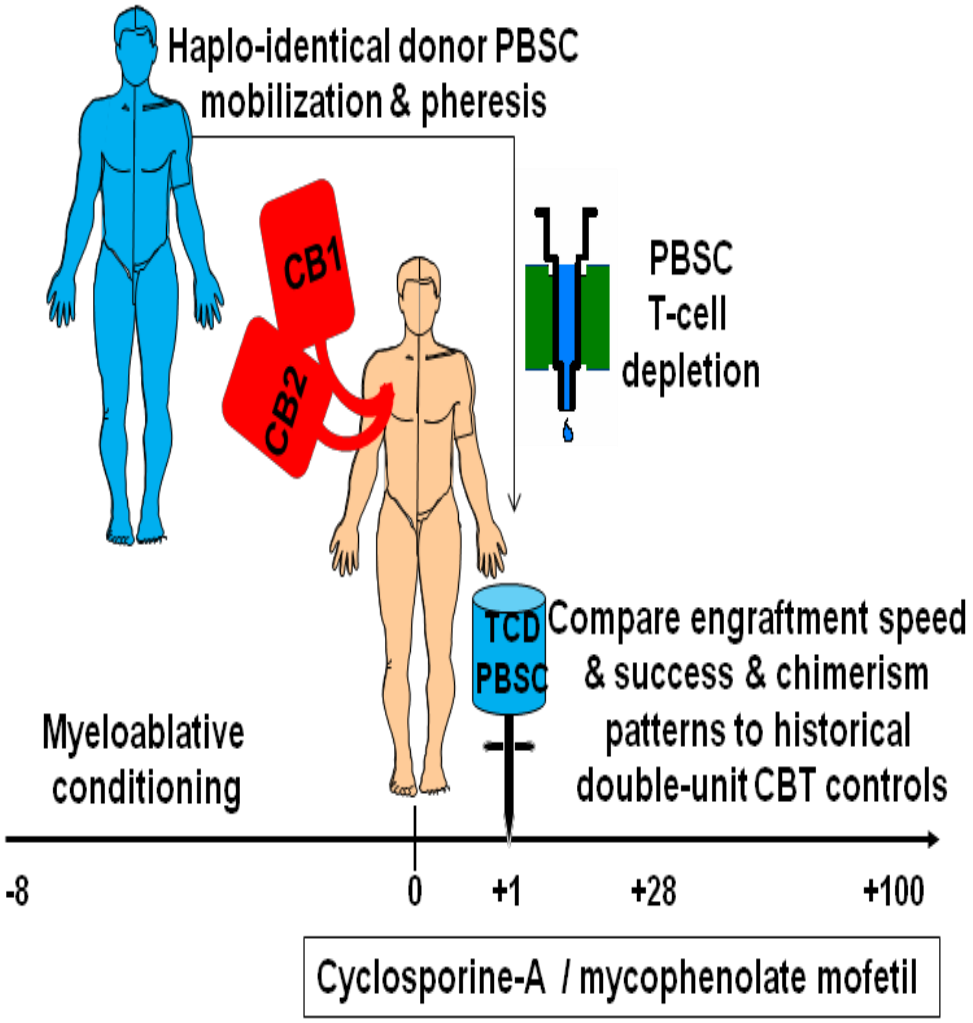
1.0 PROTOCOL SUMMARY AND/OR SCHEMA

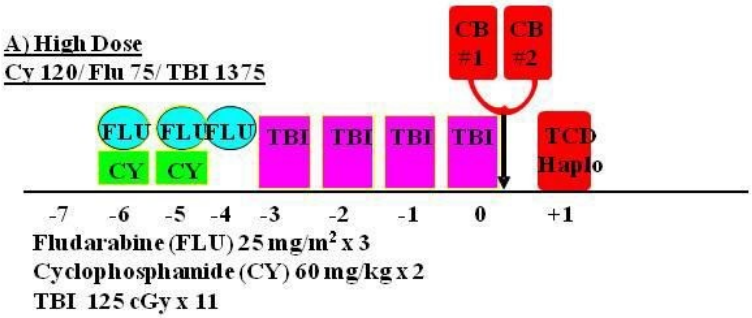
This is a phase 2 study to obtain an estimate of the speed of neutrophil recovery after myeloablative double-unit cord blood (CB) transplantation (CBT) with abrogation of prolonged cytopenia by the infusion of T-cell depleted peripheral blood stem cells (PBSC) from a haplo-identical family member. The CB graft will consist of one or two (or double) units from unrelated newborn donors to be administered on day +0 (or +1 if needed for scheduling) and haplo-identical related donors will be used to obtain PBSC for infusion on day 0 or +1. Candidates for this trial will include patients aged 2-70 years with high-risk or advanced forms of hematologic malignancies including acute myeloid leukemia (AML) or acute lymphoblastic leukemia (ALL), myelodysplastic syndrome (MDS), chronic myeloid leukemia (CML), or lymphoma for whom an allogeneic hematopoietic stem cell transplant is indicated and for whom no suitably human leukocyte antigen (HLA)-matched and readily available unrelated donor exists. For the purposes of analysis and monitoring of stopping rules, patients will be enrolled on 2 arms according to their risk for early transplant-related mortality (TRM) or relapse-related death. Arm A will include patients who are at standard risk of early post-transplant death from TRM or relapse, and Arm B will include patients who are at high risk of early post-transplant death.

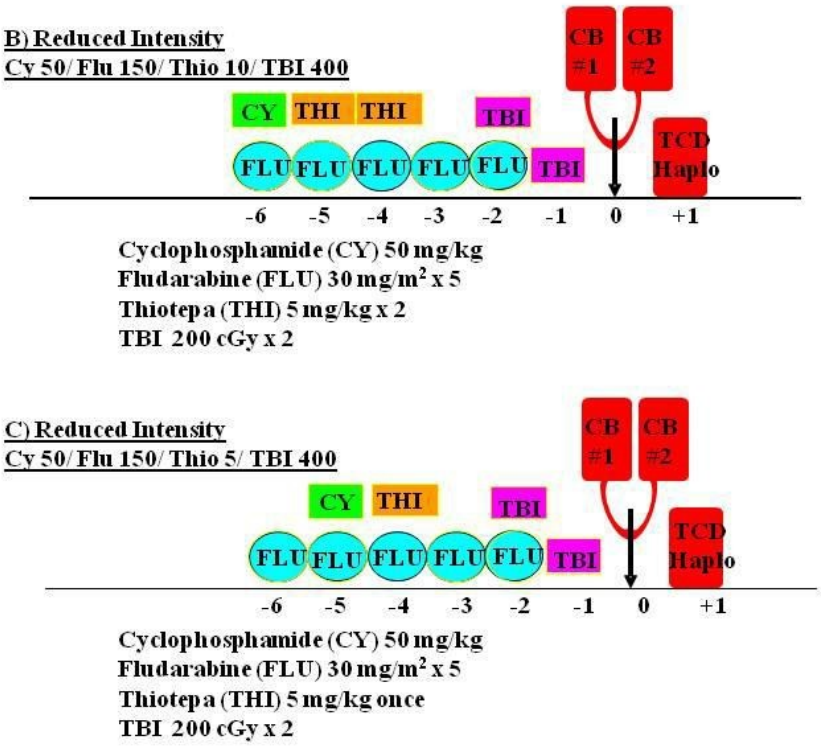
Patients must be suitable for myeloablative conditioning but this can be at high dose (cyclophosphamide 120 mg/kg, fludarabine 75 mg/m^2, and total body irradiation 1375 cGy), or reduced intensity using either 10 mg/kg or 5 mg/kg of thiotepa (cyclophosphamide 50 mg/kg, fludarabine 150 mg/m^2, thiotepa 10 mg/kg, total body irradiation 400 cGy or cyclophosphamide 50 mg/kg, fludarabine 150 mg/m^2, thiotepa 5 mg/kg, total body irradiation 400 cGy) as appropriate according to disease status, patient age, extent of prior therapy, organ function, and presence of co-morbidities. Cyclosporine-A (CSA) and mycophenolate mofetil (MMF) will be used for graft-versus-host disease (GVHD) prophylaxis. CB grafts will consist of one or two CB units 4-6/6 HLA-matched to the patient to augment graft cell dose and additional T-cell depleted PBSC from a haplo-identical donor will be infused on day 0 or +1. The aim of the haplo-identical PBSC graft is to facilitate transient engraftment and consequent abrogation of the prolonged cytopenia normally associated with CBT by providing a neutrophil and platelet bridge until CB engraftment occurs. The haplo-identical natural killer (NK) cells could also mediate additional anti-leukemia activity, especially in myeloid malignancies.

Patients will be carefully monitored for donor engraftment and count recovery, donor chimerism, incidence and severity of acute and chronic GVHD, serious infections, immune recovery, TRM, length of hospital stay, relapse as well as overall and disease-free survival. A total of 26 patients will be accrued to the standard risk arm A and 75 to the high risk arm B. Stopping rules will guard against excess toxicity according to the study arm. It is anticipated that the accrual period will last approximately 3-4 years. The primary endpoint of the study is the day to neutrophil recovery. This will be analyzed in each arm separately. Historically, the median time to neutrophil recovery for myeloablative double unit CBT patients is 25 days. A design that differentiates between population median recovery times of 25 days and 15 days will be used to assess the neutrophil recovery rate in this trial. The design has power equal to 0.90 if the population median neutrophil recovery time for patients treated under the proposed approach is 15 days; the one-sided test size is 0.10 when the median recovery time is 25 days. At the conclusion of the study a definitive assessment as to whether this approach speeds neutrophil recovery will be possible. In addition, a preliminary assessment of platelet engraftment and the efficacy of CBT plus haplo-identical PBSC will be made, and results can also be compared to historical MSKCC double-unit CBT controls.

TREATMENT SCHEMA







Cyclosporine and Mycophenolate Mofetil will be the GvHD prophylaxis for all 3 Regimens.

2.1 OBJECTIVES AND SCIENTIFIC AIMS

Data from MSKCC have shown that double-unit CBT is efficacious with 2-year progression-free survival comparable to unrelated and related donor hematopoietic stem cell (HSC) sources[1]. However, CBT is associated with delayed engraftment as compared with the transplantation of adult donor PBSC, even when using CB grafts[1]. This can contribute to increased toxicity, extended hospitalization, and increased transplantation costs. This protocol will investigate a novel treatment strategy that supplements the double-unit graft with additional haplo-identical PBSC that have been T-cell depleted with the aim that the haplo-identical cells will mediate transient engraftment and thus form a neutrophil and platelet bridge until sustained CB engraftment can be achieved. NK cells from the PBSC graft could also provide additional anti-leukemia activity.

The **primary aim** of this study is to obtain an estimate of the speed of neutrophil recovery after double-unit CBT with a T-cell depleted haplo-identical allograft.

Secondary objectives include:

- the incidence of sustained cord blood-derived neutrophil engraftment;
- the speed of platelet recovery and incidence of platelet engraftment by day 180;
- the contribution of the haplo-identical PBSC to initial and sustained engraftment by assessment of chimerism in blood and bone marrow;
- the contribution of each CB unit to initial and sustained engraftment by assessment of chimerism in blood and bone marrow;
- the incidence and severity of acute GVHD at 100 days;
- the incidence and severity of chronic GVHD at 1 year;

- the incidence of day 100 and 180 TRM;
- the length of initial hospitalization post-CBT;
- the number of days hospitalized from day 0 to day 100 post-CBT;
- immune recovery after transplant;
- the incidence of malignant relapse at 1 and 2 years;
- the probabilities of overall and disease-free survival at one and 2 years after CBT.
- graft characteristics potentially associated with engraftment.

At study completion an estimate of the efficacy of CBT with a T-cell depleted haplo-identical PBSC transplant in augmenting the speed of neutrophil engraftment will be possible.

3.0 BACKGROUND AND RATIONALE

Clinical Results with Unrelated Donor Double-Unit CBT

HSC transplantation is now recognized as an effective form of therapy for an increasing number of malignant and non-malignant disorders. To reconstitute hematopoiesis after myeloablative therapy, the transplantation of pluripotent HSCs is required. In the allogeneic setting such HSCs are traditionally obtained from the bone marrow or peripheral blood of related or unrelated adult donors. Unfortunately suitably HLA-matched allogeneic donors are not available for many patients, especially those from racial and ethnic minorities. A recent MSKCC study has shown that unrelated donor availability is limited in many patients of southern and mixed European ancestry, as well as the majority of patients of Asian, Hispanic, African, Middle Eastern backgrounds and partial non-European origins[2].

Human CB is an alternative source of HSCs that is capable of reconstituting hematopoiesis after myeloablative therapy. Known benefits of publically banked CB include: 1) rapid availability, 2) absence of donor risk, 3) absence of donor attrition, and 4) very low risk of transmissible infectious diseases such as cytomegalovirus (CMV) and Epstein-Barr virus (EBV). Furthermore, this HSC source permits the expansion of the available donor pool in targeted racial and ethnic minorities. All published studies to date have indicated that the level of HLA-match required for a successful unrelated donor CBT is less than that required for a successful unrelated donor transplant[1]. Moreover, a MSKCC study has proven that CB extends transplant access to racial and ethnic minorities due to reduced stringency of the required HLA-match (Figure 1)[2].

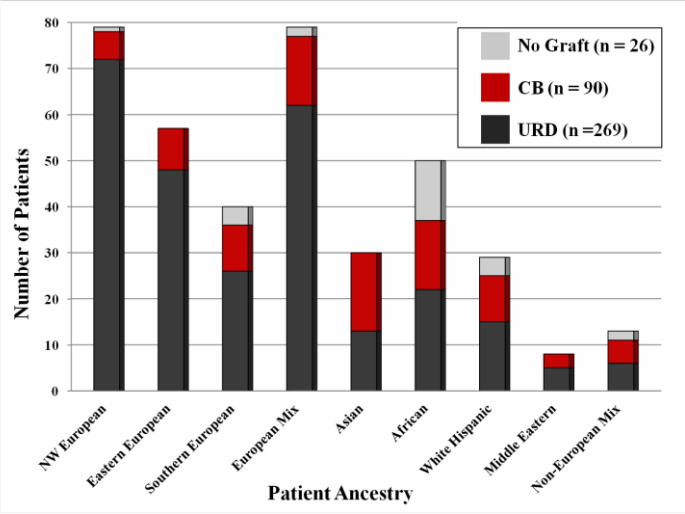


Figure 1. Stem Cell Source According to Patient Ancestry (n = 385).

Single unit CBT, however, has been associated with a significant incidence of graft failure[3,4]. Double-unit CBT has been adopted, therefore, to augment graft cell dose and has been associated with improved sustained donor engraftment as compared with historical single unit controls despite nearly all patients exhibiting sustained donor engraftment with only one of the two units of the graft (known as the dominant unit)[5-7]. Double-unit transplantation has also been associated with a reduced incidence of malignant relapse as compared with single unit CBT or adult donor transplantation[8-11]. A study from MSKCC has shown that double-unit CBT is efficacious with 2-year progression-free survival comparable to related and unrelated donor transplantation (Figure 2)[1]. In a recent series of 75 patients transplanted with double-unit CB grafts for the treatment of high-risk acute leukemia in remission or myelodysplasia with $\leq$ 5% blasts, the 2 year relapse incidence has been very low at 6% in adults and 9% in children[12]. This has translated to relatively high rates of disease-free survival at 2 years post-transplant (Figure 3). Importantly, there was no difference in the disease-free survival after high-dose and reduced intensity regimens supporting the adoption of reduced intensity CBT in patients unsuitable for high dose conditioning (Figure 4). Thus, double-unit CBT can extend transplant access to racial and ethnic minorities and achieve very promising disease-free survival in children and adults.

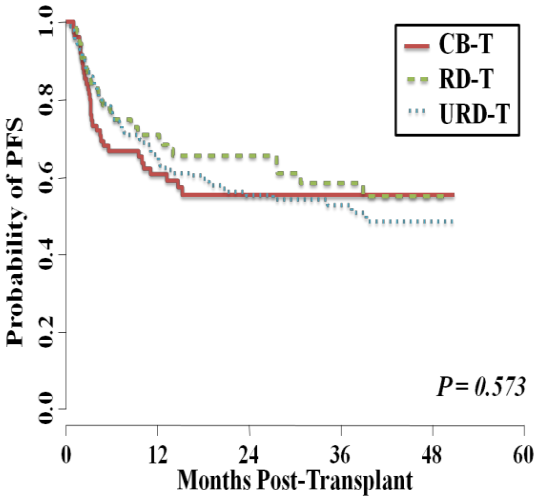


Figure 2. Post-Transplant PFS by HSC Source (n = 367).

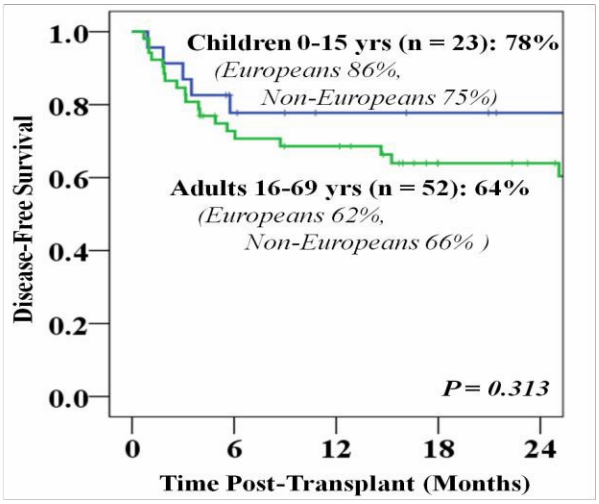


Figure 3. 2 Year DFS after Double-Unit CBT for Acute Leukemia/ MDS (n = 75).

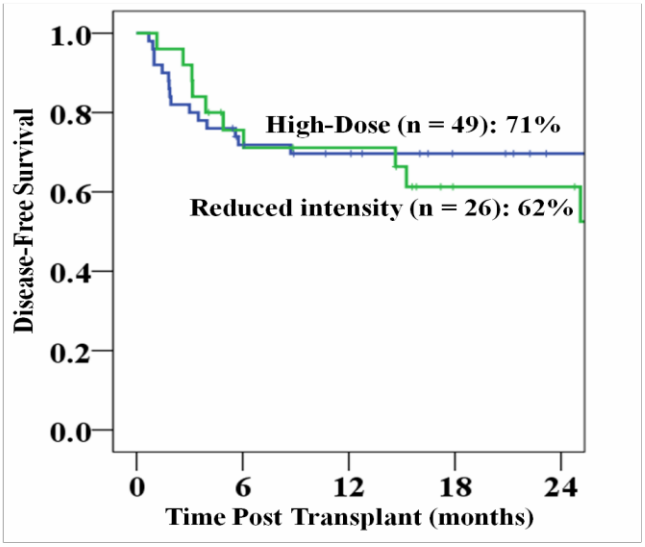


Figure 4. 2 Year DFS by Conditioning after Double-Unit CBT: High Dose vs Reduced Intensity (n = 75).

These findings support double-unit CBT as an important alternative treatment approach for high risk hematological malignancies. However, a limitation of double-unit CBT is that neutrophil recovery remains delayed even with the use of double-unit grafts. A recent review of the engraftment after myeloablative double-unit CBT (n = 80) at MSKCC demonstrated a sustained donor engraftment incidence of 94% (95% CI: 88-100) but at a delayed median of 25 days (range 13-43) (J.Barker, unpublished data, 2011). Platelet recovery was also prolonged with a cumulative incidence of 84% (95% CI: 75-92) occurring at a median of 48 days (range 30-162). As patients do not recover from the toxicity of the preparative regimen until neutrophil engraftment occurs, slow count recovery contributes to increased cost of transplantation from additional days of hospitalization, antibiotic use, narcotic use, total parenteral nutrition requirements, and transfusion dependence for red blood cells and platelets. This delayed engraftment also increases infectious complications and exacerbates pulmonary and gastro-intestinal toxicity. Most importantly, MSKCC data suggests there may be an adverse effect on survival from slow engraftment with TRM occurring in 6/32 (19%) of fast engrafters and 9/29 (31%) of slow engrafters (J.Barker, unpublished data, 2011, Figure 5).

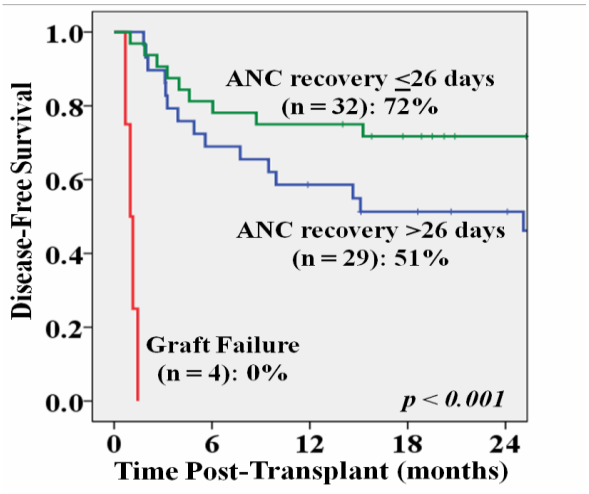


Figure 5. 2-year DFS After Myeloablative Double-Unit CBT in Adults (n = 65).

Other investigators have reported an increased TRM in engrafting patients with delayed neutrophil recovery[10]. This protocol seeks to improve the safety and tolerability of CBT by testing whether additional infusion of T-cell depleted haplo-identical PBSC can abrogate prolonged neutropenia and thrombocytopenia. These cells are capable of rapid engraftment, but are not likely to mediate sustained engraftment given they are T-cell depleted and will compete against the T-cell replete double-unit CB graft. Thus, it is hoped that the T-cell depleted haplo-identical PBSC will provide a neutrophil and platelet bridge prior to the engraftment of the double-unit CB graft at 3-4 weeks post-transplant. Optimizing the success of CBT by such an approach is important as this will significantly broaden the availability of allogeneic transplantation as a treatment modality.

Methods of Improving Neutrophil Recovery Post-transplant

Ex vivo expansion of CB has been investigated as a potential solution to the prolonged neutropenia observed after unmodified single or double-unit CBT[13,14]. However, this strategy has proven to be extremely challenging from a logistical standpoint as expansion of a portion of a single-unit graft, or one of the two units of a double-unit graft, must commence prior to the patient"s transplant admission date. Expansion is also very expensive due to the cost of supplemental GMP grade cytokines and other reagents. To date, no expansion strategy has yet proven to reliably mediate early neutrophil recovery or can easily be applied in a multi-center setting.

Fernandez et al have investigated the alternative approach of infusing a T-cell depleted haplo-identical or third party donor graft to supplement single-unit CBT[15]. Fifty-five patients with high-risk hematologic malignancies (median age 34 years and median weight 70 kg) received a single-unit CBT with a median infused total nucleated cell (TNC) dose of 2.39 x 10^7/kg with mobilized PBSC cells from a G-CSF mobilized haplo-identical (n = 38) or third-party donor that did not share haplotypes (n = 17). This graft was T-cell depleted using CD34+ cell selection. Initial predominance of the haplo or third-party donor was seen with a median time to neutrophil recovery of 10 days post-transplant and a median time to a platelet count of > 20 x 10^9/l of 32 days. Subsequently, 50/55 patients switched to CB mediated hematopoiesis as would be predicted given the CB graft was T-replete. The 5 year overall and disease-free survival were 56% and 47%, respectively.

More recently, van Besien and colleagues have investigated this approach in 45 patients with advanced hematologic malignancies[16]. The median age was 50 years and patients received reduced intensity conditioning with melphalan, fludarabine, and anti-thymocyte globulin with tacrolimus and mycophenolate mofetil immunosuppression. The median haplo-identical CD34+ cell dose was 3.5 x 10^6/kg with 0.7 x 10^4/kg CD3+ T-cells whereas the median infused TNC dose of the single-unit CB graft was 1.5 x 10^7/kg. The cumulative incidence of neutrophil engraftment was 95% (95%CI: 87-100) at day 50 with a median time to neutrophil recovery of 11 days. The cumulative incidence of platelet engraftment was 83% (95%CI: 69-97) with a median time to recovery of 19 days. In the majority of patients, early haplo-identical engraftment was subsequently replaced by durable CB mediated hematopoiesis by 100 days post-transplant, although in 6 patients there was only engraftment of the haplo-identical donor, 4 patients had engraftment with only the CB unit and 4 patients had secondary graft failure. The percentage of haplo-donor chimerism on day 100 corresponded with the infused CD34+ cell dose of the haplo-identical graft. No grade IV acute GVHD was seen. The estimated 1 year overall survival was 55% (95%CI: 39-71) and the progression-free survival was 42% (95%CI: 25-79). These results represent promising preliminary data but could be further improved upon. As no reports exist of double-unit CBT with a T-cell depleted haplo-identical graft we will pursue this strategy in an attempt to further improve upon post-transplantation engraftment and survival.

T-Cell Depletion (TCD) Using Clinimacs

MSKCC has a long history of using TCD to facilitate allogeneic transplantation with a much reduced risk of GVHD. Graft manipulation with TCD is a routine procedure for our Bone Marrow Transplant Program. In this protocol, allogeneic haplo-identical G-CSF-mobilized PBSC will be T-cell depleted by positive selection of CD34+ progenitor cells using the CliniMACS system. The CliniMACS Fractionation system requires an IND; it positively selects CD34+ progenitor cells from PBSC by immunoadsorption of cells binding an anti-CD34+ monoclonal antibody to para-magnetic beads which can then be isolated by passage through a magnetized column and released by removing the magnetic field and agitation of beads. This approach has been successfully used at MSKCC, and has provided CD34+ cell enrichment with a median CD3+ T-cell dose of only 5 x 10^3/kg in 74 recipients of adult donor allografts (G. Koehne, personal communication, October 2011).

Potential Anti-leukemic Activity of Natural Killer Cells in Haplo-identical Grafts

Haplo-identical grafts have the potential to mediate anti-malignancy effects, even if not associated with sustained engraftment of the haplo-identical donor. This is highly relevant for this protocol as patients in morphologic relapse will be included. Such patients are not usually considered for transplantation due to their high risk of relapse. The anti-leukemic effects of haplo-identical grafts are primarily mediated by NK cells. NK cells are tolerant to cells expressing self-MHC class I molecules and cytotoxic to cells lacking self-MHC class I molecules. Recognition of HLA class I ligands on potential target cells occurs through surface inhibitory receptors: KIR2DL2/3 recognize HLA-C alleles (HLA-C1 group); KIR2DL1 recognizes HLA-C alleles (HLA-C2 group); and KIR3DL1 recognizes HLA-A and HLA-B alleles with the Bw4 epitope. With expression of inhibitory KIR receptors for self-class I molecules, NK cells are "licensed" for effector function (cytokine production and cytotoxicity), and become activated against target cells lacking self-HLA class I ligands ("missing self")[17,18]. In HLA-mismatched HSC transplants, allo-reactivity of donor NK cells due to missing self recognition in the patient is the basis for a marked reduction in AML relapse and improved survival[19]. The *activating* KIR2DS1, which recognizes HLA-C2 molecules, also educates NK cells for effector function, where NK cells from HLA-C2/C2 individuals who are 2DS1-positive exhibit reduced functional capacity[20]. Therefore, both inhibitory and activating KIR receptors contribute to NK education. Because the receptors are constitutively expressed in the NK repertoire, licensed NK activity and allo-reactivity can be predicted based upon KIR and HLA genotypes with a high degree of accuracy. In this proposal, to maximize NK allo-reactivity, potential haplo-identical donors will be prioritized based on KIR/HLA where possible.

We have evaluated the impact of donor-recipient KIR-HLA genotypes for 1277 AML patients transplanted between 1989-2008 from 9-10/10 HLA-matched unrelated donors (K. Hsu, unpublished data, manuscript submitted 2012). Two activating KIR genes exhibited significant impact on relapse. Patients receiving allografts from donors positive for KIR2DS1 (n = 412) had lower relapse (HR 0.76, p = 0.02, Figure 6). Consistent with recent in vitro findings identifying its role in education of NK cells with an HLA-C1 background, HLA-C1 donors positive for KIR2DS1 had significantly better impact on relapse compared with HLA-C2/C2 donors positive for KIR2DS1 (HR 0.45, p = 0.002, Figure 7). Notably, the most potent KIR2DS1 effect was evident in an HLA-C mismatched transplant (HR 0.44, p = 0.01, Figure 8). These data suggest that KIR2DS1 provides a potent protection from relapse, and have significant implications for donor selection algorithms in that allografts from KIR2DS1 positive HLA-C1 donors have the highest anti-AML potency and that 2DS1 positive HLA-C2/C2 donors should be avoided. A clear algorithm will be used for donor selection based on KIR/HLA genotyping, applying the most current findings in NK biology to clinical practice. To maximize NK alloreactivity, potential haplo-identical donors will be prioritized based on KIR/HLA genotypes (see section 6.4).

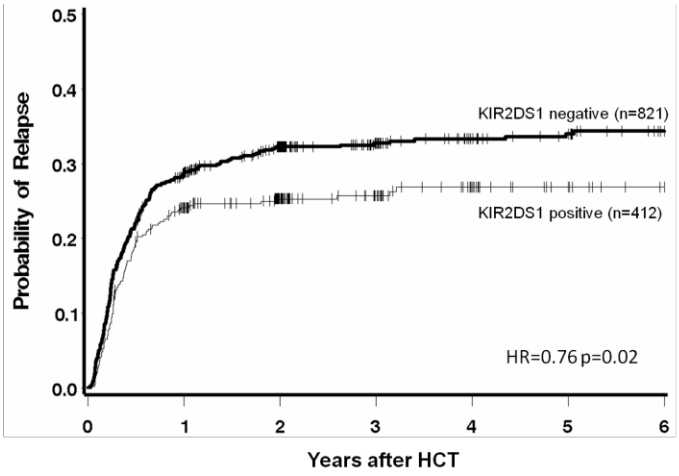
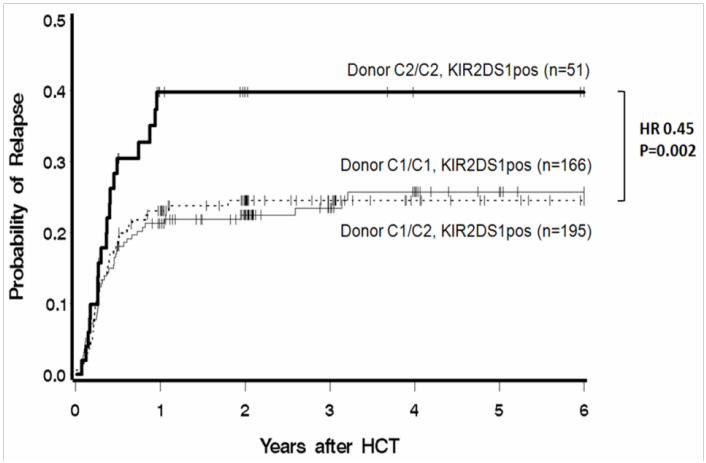


Figure 6. Donor KIR2DS1 is Associated with Lower Relapse After Unrelated Donor HSC Transplantation for AML.

Figure 7. Benefit of Donor KIR2DS1 is Most Significant in Donors with an HLA-C1 Background.



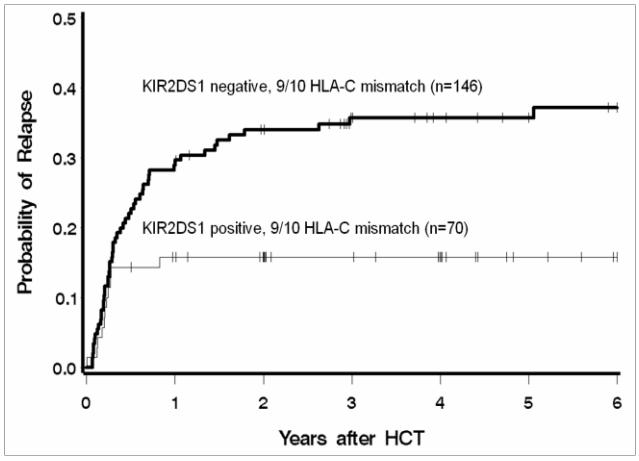


Figure 8. Potent Donor KIR2DS1-Mediated Graft-versus-Leukemia Effect in HLA-C Mismatched Unrelated Donor HSC Transplantation for AML.

Reasons to Pursue Double-Unit CBT with Haplo-identical Transplant Versus Other Transplant Strategies

An alternative treatment approach for patients with high risk hematologic malignancies would be to receive a T-cell depleted haplo-identical transplantation without CBT. However, haplo-identical transplantation in adults has been associated with a high incidence of TRM and relapse with only relatively small series being published to date with most series showing no clear plateau on the survival curve[21]. By contrast, unrelated donor double-unit CBT has become a standard alternative treatment approach at MSKCC and other centers in the United States, and a comparable disease-free survival after double-unit CBT and related and unrelated donor transplantation has been demonstrated in 2 series[1,10].

Another possibility would be to perform a single-unit CBT with haplo-identical PBSC. However, we have demonstrated variable CD34+ cell viability in CB units from public CB banks as shown in Figure 9 with poor viability units unlikely to engraft[22]. In a series of 44 double-unit CBT recipients engrafting with one unit, using a threshold of 75% (mean-2SD), all but one (43/44) engrafting units had CD34+ viability $\geq$75% and only 1/16 poor viability units engrafted (p=0.0006). Poor viability correlated with lower colony-forming-unit content (p = 0.02). This raises the possibility that one of the reasons why double-unit CBT is effective is that it increases the chance of infusing at least one unit of high quality and thus with engraftment potential.

An additional reason why double-unit CBT has been associated with augmented engraftment is due to the increased dose of total nucleated cells. The MSKCC study of Avery et al has shown that the speed and success of engraftment is associated with the total TNC and CD3+ T-cell doses of both units combined (Figure 10) as well as the CD34+ cell dose of the dominant unit[7]. This suggests that the non-engrafting unit is facilitating the engraftment of the dominant unit despite the fact that it does not contribute to engraftment itself, and that this effect is dose dependent. Single-unit CBT plus TCD haplo-identical transplantation has been associated with a risk of graft failure of the CB, and patients with sustained engraftment of the haplo-identical cells or failure of both stem cell sources have a poor prognosis[16].

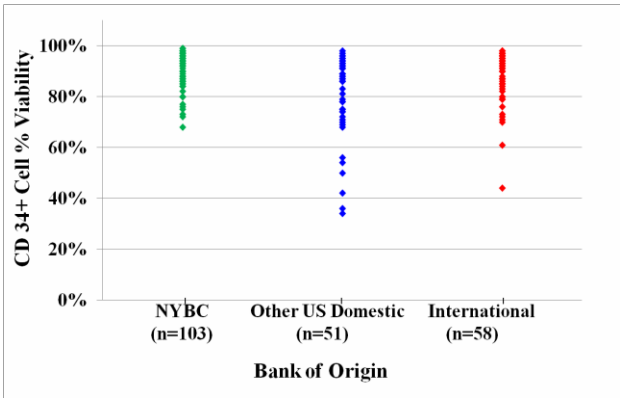


Figure 9. Variable CD34+ Cell Viability Reflecting Variable Quality of CB Units from Public Banks.

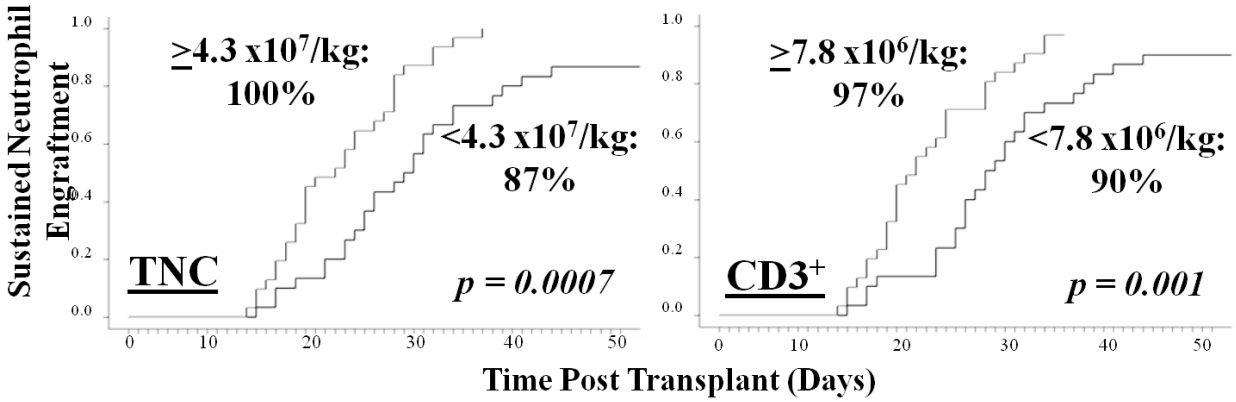


Figure 10. Total Graft Cell Dose & Neutrophil Engraftment After Myeloablative Double-Unit CBT (n = 61).

Finally, the most compelling reason to use double-unit CBT is that multiple analyses have suggested that double-unit CBT protects against malignant relapse[8-11]. This is especially important in adults who receive reduced intensity conditioning. It is unknown whether such benefits would be derived from single-unit CB with haplo-identical PBSC transplantation. As survival after double-unit CBT has been comparable to that of HSC transplantation from adult donors, this protocol will use of double-unit grafts for the vast majority of patients and investigate the addition of haplo-identical PBSC to abrogate the prolonged cytopenia and potentially facilitate an additional NK-mediated anti-leukemia effect. Most importantly, our approach will also facilitate comparison of the results with those of MSKCC double-unit CBT historical controls. However, single unit CBT plus haplo-identical PBSC infusion will be considered in the occasional pediatric patient with a large well matched single unit from a good quality bank or the rare patient with only one unit of suitable match and dose characteristics.

4.1 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.2 Design

This is a 2 arm phase 2 study to obtain an estimate of the speed of neutrophil recovery after myeloablative CBT with abrogation of prolonged cytopenia by infusion of haplo-identical family member T-cell depleted PBSC in patients with high-risk or advanced hematologic malignancies. 101 patients will be recruited over 4 years and transplant outcomes will be assessed as per Section 2.0. Arm A (n = 26) will include eligible patients who are at standard risk of early post-transplant death from TRM or relapse, whereas Arm B (n = 75) will include eligible patients who are at high risk for early death. Stopping rules will guard against excess toxicity as evidenced by graft failure, severe GVHD, and high day 100 death rates from TRM or relapse according to risk group (i.e. if in Arm A or B, see Section 14). Problems with engraftment, severe GVHD, TRM, early death due to relapse and other adverse experiences will be carefully monitored by the MSKCC BMT research data managers and reported to the Principal Investigator. Outcomes will be analyzed in each study arm separately and at the conclusion of the study the results of study patients can also be compared to MSKCC double-unit CBT historical controls.

4.3 Intervention

This is a two arm phase 2 study. In the majority of patients the CB graft will consist of double units from two unrelated newborn donors. However, rare patients may be able to proceed with a single CB unit. The CB graft (administered day 0 or day 0 and +1) will be combined with a PBSC graft obtained from a haplo-identical related donor and this will be administered on day 0 or +1. Candidates will include patients aged 2-70 years with high-risk or advanced hematologic malignancies for whom an allogeneic HSC transplant is indicated and for whom no suitably HLA-matched and readily available donor exists. Patients must be suitable for myeloablative conditioning but this can be at high dose or reduced intensity as appropriate. GVHD prophylaxis will include CSA and MMF. CB grafts will consist of partially HLA-matched CB unit(s) and additional T-cell depleted haplo-identical PBSC will be infused on day 0 or +1. The aim of the haplo-PBSC graft is to achieve transient engraftment that will abrogate the prolonged cytopenia usually associated with CBT until sustained CB engraftment can occur. The haplo-identical NK cells could also mediate additional anti-leukemia activity.

5.1 THERAPEUTIC/DIAGNOSTIC AGENTS

5.2 Cyclophosphamide (Cytoxan®, Neosar®)

Supplied as: 200 mg, 500 mg, 2000 mg vials
Reconstitution directions: add sterile water for injection to yield a final concentration of 20 mg/ml.
Storage and stability:
1. Store vials at room temperature.
2. Refrigerated: prepare infusion in D5W, stable for 28 days.
3. Room temperature: prepare infusion in D5W: stable for 48 hours.
Preparation:
1. Standard iv fluid: D5W.
2. Final concentration range up to: 20mg/ml.
3. IV piggyback volume: for doses < 1200mg/m^2, infuse in 25cc D5W; for doses> 1200mg, infuse as straight drug.
Clinical considerations:
1. Hydration: as per MSKCC guidelines for BMT patients receiving > 3000 mg/m^2.
2. Emetic potential: high and delayed.
3. Supportive medications: anti-emetics and mesna.
Toxicities: see Section 11.0.
Incompatibilities: do not administer with other drugs.

5.3 Mesna (Mesnex®)

Supplied as: 200 mg/2 ml ampule, 1 gram/10 ml multi-dose vials
Reconstitution: not applicable.
Storage and stability: Store vials at room temperature. Multi-dose vials may be stored and used for up to 8 days after initial entry. Infusions prepared in D5W are stable for 48 hours when stored under refrigeration or at room temperature.
Preparation:
1. Must be diluted prior to infusion
2. Standard IV fluid: D5W.
3. IV piggyback volume: 50ml D5W
Dosage and administration: 100% of total cyclophosphamide dose divided into 3 doses administered at 30 minutes prior to and 4 and 8 hours after the start of the chemotherapy. Given as IVPB over 15 minutes.

Clinical Considerations: functions as an uroprotectant to prevent hemorrhagic cystitis.
Toxicities: none.

5.4 Fludarabine phosphate (Fludara®)

Supplied as:50mg vial
Reconstitution directions: add 2ml of sterile water for injection to a 50mg vial; yields a final concentration of 25 mg/ml.
Storage and stability:
1. Store vials under refrigeration.
2. Refrigerated: prepare infusion in D5W; stable for 16 days.
3. Room temperature: prepare infusion in D5W; stable for 16 days.
Preparation:
1. Standard iv fluid: D5W.
2. Final infusion concentration range: up to 10mg/ml.
3.IV piggyback volume: 50 cc.
Clinical considerations:
1. Hydration: during 500 cc saline.
2. Emetic potential: low.
Supportive medications: none.
Toxicities: see Section 11.0.
Incompatibilities: acyclovir, amphotericin B, chlorpromazine, daunorubicin, ganciclovir, hydroxyzine, miconazole, prochlorperazine.

5.5 Thiotepa (Thioplex®)

Supplied as:15 mg, 30 mg, powder for reconstitution
Reconstitution directions: Reconstitute each vial to 10 mg/mL. Solutions for infusion should be diluted to a concentration ≥5 mg/mL in 5% dextrose or 1, 3, or 5 mg/mL in 0.9% sodium chloride injection. Filter through a 0.22 micron filter prior to administration.
Storage and stability:
1. Store intact vials under refrigeration (2°C to 8°C). Protect from light.
2. Reconstituted solutions (10 mg/mL) are stable for up to 28 days under refrigeration (4°C to 8°C) or 7 days at room temperature (25°C).
3. Solutions for infusion in D_5W (≥5 mg/mL) are stable for 14 days under refrigeration (4°C) or 3 days at room temperature (23°C).
4. Solutions for infusion in NS (1, 3, or 5 mg/mL) are stable for 48 hours under refrigeration (4°C to 8°C) or 24 hours at room temperature (25°C). Solutions in NS at a concentration ≤0.5 mg/mL are stable for <1 hour.
Clinical considerations:
Hydration: NA
Emetic potential: low.
Supportive medications: none.
Toxicities: see Section 11.0.

5.6 Total Body Irradiation (TBI)

The intensity of the TBI will be determined by the treating BMT physician who will select a conditioning regimen of either high dose or reduced intensity conditioning as described in Section 9.1. Treatment planning begins with a simulation. Patients will receive high-dose treatment (a total of 1375 cGy over 4 days in 11 fractions, or a total of 1200 cGy over 4 days in 8 fractions if unable to tolerate 11 fractions) or reduced intensity (a total of 400 cGy over 2 days in 2 fractions). Lung

shielding will be done in recipients of high dose radiation (≥ 1200 cGy) as per MSKCC standard practice. Pre-transplant radiation boosts to the testis or other areas are permitted as appropriate for the underlying diagnosis and will be decided in consultation with Radiation Oncology clinicians. Patients are treated in a standing position and the treatment takes 20-30 minutes. Toxicities are outlined in Section 11.0.

5.7 Cyclosporine

Supplied as: 50 mg/ml; 5 ml ampule (protect from light)
Reconstitution: N/A
Indications: Immunosuppressant used in the allogeneic transplantation.
Storage and Stability: Prepare in a glass bottle only. Stability is 72 hours under refrigeration or at room temperature.
Preparation:
1. Dilute in D5W or NS to make a 2.5 mg/ ml solution.
2. Infuse slowly over approximately 1-4 hours.
Clinical Considerations:
1. Patients should be under close observation for possible allergic manifestations including flushing, dyspnea and wheezing, blood pressure changes and tachycardia.
2. Prior to infusion solution should be inspected for particulate matter and discoloration.
3. Other nephrotoxic agents will increase the risk of nephrotoxicity.
4. Plasma concentrations of cyclosporine may be affected by the following drugs:
Increased cyclosporine levels: ketoconazole, erythromycin, cimetidine, calcium channel blockers, fluconazole, itraconazole, norfloxacin, imipenem/ cisplatin.
Decreased cyclosporine levels: rifampin, phenytoin, phenobarbital, imipenem/ cisplatin.
5. The target serum level of 200- 400 is desirable; 600 is considered toxic.
6. Renal and hepatic function should be monitored closely.
Toxicities: see section 11.0
Incompatibilities: Do not co-administer with any drug.

5. 7 Mycophenolate Mofetil (CellCept®)

Supplied as: 500 mg vial of powder for reconstitution.
Reconstitution: reconstitute each 500 mg vial with 14 ml of D5W only. Gently shake to dissolve.
Storage and Stability: Store at 15 -30°C. Drug compatible with D5W only. A final concentration of 6mg/ ml must be achieved prior to administration. Reconstituted vials and IV preparations are stable for up to 4 hours after preparation.
Preparation:
1. Reconstitute each 500 mg vial with 14 ml of D5W.
2. Gently shake the vial to dissolve the drug.
3. Drug must be further diluted to a final concentration of 6 mg/ml. A 1000 mg dose should be placed in 140 ml of D5W.
4. Mycophenolate Mofetil vials are stable for 4 hours at room temperature after reconstitution.
5. Doses of mycophenolate may begin infusion up to 4 hours after initial reconstitution.
Clinical Considerations: administer only with D5W over at least 2 hours. Mycophenolate is mutagenic, carcinogenic, and teratogenic. Precautions must be taken when handling this product. If medication comes in contact with skin, wash thoroughly with soap and water.
Toxicities: see section 11.0
Incompatibilities: Only compatible with D5W.

5.8 Filgrastim/ Granulocyte-Colony Stimulating Factor (Neupogen®)

Supplied as 1 ml vial (300 mcg) and 1.6 ml vial (480 mcg); 300 mcg/0.5 ml pre-filled syringe; 480 mcg/0.8 ml pre-filled syringe.
Store at 2-8°C. Do not freeze. Avoid shaking. May reach room temperature for 24 hours prior to use.
Preparation:
1. For IV infusion, dilute filgrastim in 25-50 ml D5W.
2. The minimum concentration must not be less than 5 mcg/ml.
3. If the final concentration is between 5-15 mcg/ml, albumin 2 mg/ml must be added prior to addition of the drug.
4. Stability (IV) once diluted in 25-50 ml of D5W, filgrastim is stable for 7 days.
5. Stability (plastic syringe): filgrastim is stable for two weeks in BD 1 ml plastic TB syringes at 2-8°C.
Dose: ≤ 60 kg = 300 mcg daily subcutaneously; > 60 kg = 480 mcg subcutaneously daily.
Clinical Considerations: If administered as an intermittent IV infusion, give via an infusion control device over a 15- 30 minute period.
Incompatibilities: The drug may precipitate in Normal Saline. Do not mix with any other drugs.

5.9 Cord Blood (CB) and Haplo-identical PBSC

CB units are processed to remove excess plasma; most banks also deplete red blood cells. They are tested for sterility, HLA-typed, cryopreserved and stored. The CB units will be thawed in the Cytotherapy Laboratory as per standard operating procedures. CB units should be infused intravenously via a central venous catheter according to standard MSKCC Nursing guidelines.

Haplo-identical PBSC will be collected from a family member after G-CSF mobilization. TCD will be done by positive selection of CD34+ cells using CliniMACS. The CliniMACS Fractionation system requires an IND; it positively selects CD34+ progenitors by immunoadsorption of cells to paramagnetic beads. Cells are isolated by passage through a magnetized column and released by agitation of the beads.

6.1 CRITERIA FOR SUBJECT ELIGIBILITY

6.2 Subject Inclusion Criteria

The criteria below determine eligibility for the protocol.

Note: protocol eligible patients according to the criteria outlined below will then be divided according to age, diagnosis, performance status, organ function, prior transplantation, hematopoietic cell transplant comorbidity index (HCT-CI)[23], and CB TNC dose into those who are at standard risk (Arm A) or high risk (Arm B) for early post-transplant death for the purposes of applying stopping rules and outcome analysis.

Protocol eligibility criteria are:

- Age:
  - 2 - 70 years.
- Diagnosis of severe aplastic anemia: eligibility to be discussed with PI and Service Chief. Such patients will be assessed in Arm B.
- Diagnosis of high risk hematological malignancy:
  - Any acute leukemia in first complete remission (CR) considered at high risk for relapse, or second or third CR, or relapse/refractory less than 10% blasts in bone marrow, or aplasia post-therapy. This includes de novo acute leukemia or acute leukemia that is therapy related or arising from an antecedent hematologic disorder including

myelodysplasia (MDS), chronic myeloid leukemia (CML) or other myeloproliferative disorder.
  - Juvenile myelomonocytic leukemia (JMML) in CR, or relapse with less than 10% bone marrow blasts.
  - CML with tyrosine kinase inhibitor failure in chronic or accelerated phase or evolved to acute leukemia (blast crisis, see above).
  - MDS or other myeloproliferative disorder with life-threatening cytopenia(s), and/or red blood cell or platelet transfusion dependence, or patients with aplasia, or patients with excess blasts less than 10% blasts in the bone marrow at work-up.
  - Aggressive lymphoma**:** patients in CR1 with disease at high risk of relapse or CR2-3..
  - Indolent lymphoma or chronic lymphocytic leukemia (CLL): any disease status provided any transformed component is in CR.
  - Hodgkins lymphoma that is primary refractory or relapsed not suitable for other therapy and in PR or CR or small volume stable disease.
- Performance status:
  - Karnofsky score ≥70 or Lansky score ≥70.
- Organ function:
  - Resting left ventricular ejection fraction (LVEF) ≥ 50%.
  - Spirometry (FEV_1 and FVC) & corrected DLCO ≥ 50% predicted. In small children use history and physical and CT scan to determine pulmonary status.
  - Total bilirubin ≤ 1.5 mg/dL (unless benign congenital elevated bilirubin); ALT ≤ 3 x upper limit of normal (ULN).
  - Calculated creatinine (calc. creat.) clearance ≥ 60 ml/min.
  - Albumin ≥ 3.0.

- Graft:
  - Cryopreserved dose will be ≥ 1.5 x 10^7 TNC/kilogram in each unit for double unit CB grafts. This will be the CB graft for the majority of patients.
  - In select patients with access to CB units that have high TNC (> 5.0 x 10^7/kg), and are from good quality CB banks a single unit could be considered with a back-up CB unit on standby.
  - In select patients who have a very poor search and only have one suitable CB unit available, this unit could be given as a single unit. This unit must have a TNC ≥ 2.0 x 10^7 TNC/kilogram and a CD34+ cell dose ≥1.5 x 10^5 CD34+/kilogram.
  - Haplo-identical donors who are 5/10 or better but not HLA-identical will be used as outlined in section 6.4.

Assignment of conditioning intensity (high dose vs reduced intensity) will be based on patient disease status, age, extent of prior therapy, organ function and presence of significant co-morbidities as outlined in Section 9.2.

For the purposes of analysis (not assignment of preparative regimen), patients will be assigned to Arms A and B as summarized below according to their risk of early post-transplant death.

Eligible patients who fulfill **all** of the following criteria will be assigned to risk **Arm A:**

	Arm A: Standard Risk for Early Post-Transplant Death
Age	2-49 years
Diagnosis	Any acute leukemia in CR1 - CR2 (includes therapy-related and arising from MDS or myeloproliferative disease).

	JMML in CR. CML with TKI failure & < 5% blasts. MDS with < 5% blasts at work-up. Lymphoma (including CLL) CR1-2.
Performance Status	Karnofsky ≥80; Lansky ≥80.
Organ Function	Resting LVEF ≥ 60%. Spirometry (FEV^1 and FVC) & corrected DLCO ≥ 80% predicted. Total bilirubin normal; ALT normal-1.4 x ULN. Calc. creat. clearance ≥ 70 ml/min.
Prior HSC Transplant	No
HCT-CI score[23]	0-2
Pre-thaw TNC Dose	Each unit ≥ 2.0 x 10^7/kg.

Eligible patients who meet **any** of the following criteria will be assigned to risk **Arm B**:

	Arm B: High Risk for Early Post-Transplant Death
Age	50-70 years
Diagnosis	Any acute leukemia in relapse/ refractory disease in BM or circulating blasts or CR3 or aplasia. JMML not in CR. MDS with aplasia or ≥ 5% blasts. Lymphoma (including CLL) with disease other than CR1-2. Severe myelofibrosis of the bone marrow
Performance Status	Karnofsky 70; Lansky 70
Organ Function	Resting LVEF 50-59%. Spirometry (FEV^1 and FVC) & corrected DLCO 50-79% predicted. Total bilirubin 1.1-1.5 mg/dL; ALT 1.5-3 x ULN. Calc. creat. clearance 60-69 ml/min.
Prior HSC Transplant	Yes
HCT-CI score[23]	3 or higher
Pre-thaw TNC Dose	Either or both units 1.5-1.9 x 10^7/kg.

6.3 Subject Exclusion Criteria

- Active CNS leukemia.
- Any acute leukemia (including prior myelodysplasia or CML blast crisis) with morphologic relapse or persistent disease ≥ 10% blasts in the BM, or doubling of the blasts in the blood in the 2 weeks preceding admission, or need for hydroxyurea in the 2 weeks prior to admission, or uncontrolled extra-medullary disease.
- Two prior stem cell transplants of any kind.
- One prior autologous stem cell transplant within the preceding 12 months.
- One prior allogeneic stem cell transplant within the preceding 24 months.
- Prior radiation therapy with 400cGy or more of TBI. If 200 cGy of prior TBI then only 400 cGy of TBI on this protocol is permitted..
- Uncontrolled viral, bacteria or fungal infection at time of study enrollment.
- Sero-positive or NAT positive for HIV.
- Females who are pregnant or breast feeding.

- Patient or guardian unable to give informed consent or unable to comply with the treatment protocol including appropriate supportive care, follow-up, and research tests.

6.4 Cord Blood Grafts

Units will be selected based on the HLA-match to the patient and individual cell doses of the units according to current MSKCC unit selection criteria. HLA-testing will be done using molecular techniques. The standard cord blood graft for this protocol will consist of 2 units as a double unit graft although single units are permitted. Each unit will be at least 4 of 6 HLA-A, -B antigen and -DRB1 allele matched with the recipient. Each unit of a double unit graft will have a cryopreserved dose of at least 1.5 x 10^7 TNC/recipient body weight (TNC/kg). In the occasional patient with a large well matched good quality single unit or the rare patient with only one unit of suitable match and dose characteristics the cord blood graft can consist of a single unit as described in section 6.1.

6.5 Haplo-identical Donor Inclusion Criteria

- A HLA-haplo-identical related donor will be selected as available as per standard MSKCC Adult BMT guidelines. Mismatched family members who are matched at more than 5 of 10 HLA-loci are permitted. Factors to be taken into account when selecting a haplo-identical donor will include donor age, weight, health status and comorbidities, compliance, venous access, recipent donor specific HLA-antibody status, and NK cell alloreactivity.
- The donor must meet criteria outlined in the FACT-approved SOP for "DONOR EVALUATION AND SELECTION FOR ALLOGENEIC TRANSPLANTATION" in the Blood and Marrow Transplant Program Manual, document E-1 (see attached, or link to URL: (https://one.mskcc.org/sites/pub/corp/bmt/Documents/D2_SOP_Donor%20Selection%20and%20Evaluation_04_2015.pdf).
- The donor must have adequate peripheral venous catheter access for leukapheresis or must agree to placement of a central catheter.
- The donor must be >25 kg in weight.

6.6 Haplo-identical Donor Exclusion Criteria

- Evidence of active infection (including active urinary tract infection, or upper respiratory tract infection) or evidence of viral hepatitis exposure on screening unless only HbsAb+ and HBV DNA negative.
- Medical or physical reason which makes the donor unlikely to tolerate or cooperate with growth factor therapy and leukapheresis.
- Factors which place the donor at increased risk for complications from leukapheresis or G-CSF therapy (e.g., active autoimmune disease, sickle cell trait, symptomatic coronary artery disease requiring therapy).
- Pregnancy (positive serum or urine β-HCG) or breastfeeding. Women of childbearing age must avoid becoming pregnant while on the study.

7.0 RECRUITMENT PLAN

Adult and pediatric patients will be considered for this therapy and recruited as appropriate according to their diagnosis, availability of alternative therapies, and availability of appropriate cord blood grafts and related haplo-identical donors as described in the protocol eligibility. Patients who fulfill the eligibility criteria as listed in Section 6.0 will be recruited by a BMT attending. Informed

consent will be obtained by one of the participating investigators authorized to obtain consent. After consent is obtained, confirmation of patient eligibility will be done by the Clinical Trials Office.

This protocol will take due notice of NIH/ADAMHA policies concerning inclusion of women and minorities in clinical research populations. We expect that the study population will be fully representative of the range of patients referred for transplant without exclusion as to age, gender, or ethnic background within the limits of transplant eligibility and of being able to identify a suitable CB graft and a suitable haplo-identical related donor. Pregnant women are excluded from participation in this study.

8.1 PRETREATMENT EVALUATION

8.2 Pretreatment evaluation of the patient

1. Standard clinical pretreatment evaluations

The following tests must be performed as per Adult BMT standard of care:

a. Within 30 days prior to admission:
   i. Complete history, review of systems, physical exam (including performance status).
   ii. CBC with differential, comprehensive metabolic panel including albumin, LDH, PT/ PTT.

b. Within 45 days prior to admission:
   i. Bone marrow aspirate (trephine core if clinically required) for morphology, and special studies (surface markers, cytogenetics, FISH and molecular studies) as warranted for documentation of disease status and bone marrow morphology.

c. Prior to admission standard workup studies per Adult BMT Program including:
   i. Spinal or intra-Ommaya tap for evaluation of evidence of CNS leukemia as appropriate if patients with acute leukemia are at risk for CNS disease.Red blood cell type and screen (ABO blood type)..
   ii. EKG.
   iii. Echocardiogram or MUGA scan with measurement of left ventricular ejection fraction.
   iv. Radiographic studies if clinically indicated for diagnosis.
   v. Chest CT scan without contrast to exclude occult fungal infection prior to transplant (unless CT with contrast required for disease assessment eg NHL).
   vi. Pulmonary function testing including DLCO. Clinical and radiological assessment can substituted in small children.
   vii. Testing for CMV (IgG and IgM), HIV-1/2, HTLV-1/2, toxoplasmosis, Hepatitis B (surface antigen, surface antibody), Hepatitis C antibody, Herpes Simplex, Herpes Zoster, Epstein Barr Virus, and syphilis. Pregnancy test for females of childbearing age (serum or urine HCG).
   viii. Peripheral blood from the patient should be submitted to the Diagnostic Molecular Pathology (DMP) Laboratory for future chimerism studies.
   ix. HLA antibodies to the American Red Cross.

Note if prior serology testing has documented sero-positivity for an infection such as CMV or EBV it does not need to be repeated during the pre-transplant workup.

2. **Pretreatment Protocol Research Tests**
    a. Prior to conditioning:
        i. Research labs: approximately 30cc (green and red top tubes) should be sent to the research laboratories as part of correlative laboratory studies.

9.1 TREATMENT/INTERVENTION PLAN

9.1 General and Assignment of Conditioning Regimen

Patients will be admitted to the Pediatric or Adult Bone Marrow Transplant Unit. Patients will be maintained in reverse isolation as per the BMT clinical care guidelines. Prior to the administration of the pre-transplant conditioning, a double or triple lumen central venous catheter will be inserted.

The conditioning regimen will be selected based on the patient age as well as disease status, extent of prior therapy, organ function and presence of significant co-morbidities. Note that:

- Patients < 30 years are potentially eligible for high dose or reduced intensity regimens i.e. Regimens A), B) or C) and decision should be made based on comorbidity status.
- Patients 30-59 years are eligible for regimens B) or C) and choice will be made based on comorbidity status.
- Patients aged 60 - 70 years and patients with HCT-CI score of 5 or higher are only eligible for regimen C).

9.2 Treatment Schedule and Conditioning

Regimen A) Cy 120/ Flu 75/ TBI 1375: Fit patients < 30 years

Patients most suitable for **high-dose conditioning** will receive:

Day	Treatment
-7	Admission
-6	Fludarabine 25 mg/m^2 IV Cyclophosphamide 60 mg/kg IV (+Mesna)
-5	Fludarabine 25 mg/m^2 IV Cyclophosphamide 60 mg/kg IV (+Mesna)
-4	Fludarabine 25 mg/m^2 IV
-3	TBI (125 cGy) x 3* Start CSA and MMF
-2	TBI (125 cGy) x 3*
-1	TBI (125 cGy) x 3*
0	TBI (125 cGy) x 2* then CBT**
Day 0 or +1	Administer haplo-identical PBSC

* Can be given as 150 cGy per fraction BID for 4 days for a total of 1200 cGy if not able to tolerate TID dosing.

** If haplo-identical cells are given on day 0 and the CB units need to be split, CB can be given on day +1.

- Fludarabine: 25 mg/m^2/day IV over approximately thirty minutes x 3 days (days -6, -5 and -4) for a total dose of 75 mg/m^2 . Dose should be adjusted if the patient is > 125% ideal body weight (IBW) and calculated on adjusted body weight per MSKCC guidelines.
- Cyclophosphamide: 60 mg/kg/day IV over 30-60 minutes x 2 days (days -6 and -5) per MSKCC guidelines with high volume fluid flush and Mesna after the fludarabine is complete. Cyclophosphamide dose should be adjusted if the patient is > 125% ideal body weight (IBW) and calculated on adjusted body weight per MSKCC guidelines. High volume fluids should commence approximately 12 hours prior to drug and continue until 24 hours after the second dose. Rate should be per MSKCC standard of care (as documented on website under "for BMT patients receiving ≥ 3000 mg/m^2). Use intravenous diuretic (Lasix^{TM}) as required to maintain fluid balance.
- Mesna: Dose is 100% of the total daily cyclophosphamide dose divided into 3 doses (20 mg/kg/dose) and administered at 30 minutes prior to and 4 and 8 hours after the start of Cyclophosphamide on days -6 and -5. Give as IVPB over 15 minutes. Pediatric patients can receive the same dose as continuous infusion.
- Total Body Irradiation: 125 cGy x 11 doses (TID on days -3, -2, -1 and BID on day 0) for a total TBI dose of 1375 cGy. Patients unable to tolerate a TID dosing schedule for TBI can receive 150 cGy BID x 8 doses for a total of 1200 cGy.

Regimen B) Cy 50/ Flu 150/ Thio 10/ TBI 400: Fit patients 30-59 years or patients < 30 years not suitable for high dose conditioning.

Patients most suitable for **reduced intensity conditioning** will receive:

Day	Treatment
-7	Admit and line placement
-6	Fludarabine 30 mg/m^2 IV Cyclophosphamide 50 mg/kg IV
-5	Fludarabine 30 mg/m^2 IV Thiotepa 5 mg/kg IV
-4	Fludarabine 30 mg/m^2 IV Thiotepa 5mg/kg IV
-3	Fludarabine 30 mg/m^2 IV Start MMF and CSA IV
-2	Fludarabine 30 mg/m^2 IV, TBI 200 cGy
-1	TBI 200 cGy
0	CBT*
Day 0 or +1	Infuse haplo-identical PBSC

** If haplo-identical cells are given on day 0 and the CB units need to be split, CB can be given on day +1.

- Fludarabine: 30 mg/m²/day should be administered as per MSKCC guidelines in the morning over approximately 30-60 minutes on days -6 to -2 (5 doses). Fludarabine dose should be calculated based upon adjusted body weight if the patient is ≥ 125% ideal body weight.
- Cyclophosphamide: 50 mg/kg IV will be given for one dose. It should be administered as per MSKCC guidelines on day -6 after the fludarabine is complete. Cyclophosphamide dose should be adjusted if patient is ≥ 125% ideal body weight (IBW) and should be calculated on adjusted body weight per MSKCC standard of care guidelines. Fluids should be per MSKCC standard of care with diuretics as required to maintain fluid balance.
- Thiotepa: 5 mg/kg/day IV given as a 4 hour infusion on days -5 and -4. Thiotepa dose will be calculated based upon adjusted body weight if the patient is ≥ 125% ideal body weight.

- Total Body Irradiation: 200 cGy per dose on days -2 and -1 (2 doses). .

Regimen C) Cy 50/ Flu 150/ Thio 5/ TBI 400: patients 60-70 years or patients < 60 years not suitable for conditioning of higher intensity including patients with HCT-CI score of 5 or higher.

Patients most suitable for **reduced intensity conditioning with thiotepa 5 mg/kg** will receive:

Day	Treatment
-7 or -6	Admit and line placement
-6	Fludarabine 30 mg/m^2 IV
-5	Fludarabine 30 mg/m^2 IV Cyclophosphamide 50 mg/kg IV
-4	Fludarabine 30 mg/m^2 IV Thiotepa 5mg/kg IV
-3	Fludarabine 30 mg/m^2 IV Start MMF and CSA IV
-2	Fludarabine 30 mg/m^2 IV, TBI 200 cGy
-1	TBI 200 cGy
0	CBT
Day 0 or +1	Infuse haplo-identical PBSC

** If haplo-identical cells are given on day 0 and the CB units need to be split, CB can be given on day +1.

- Fludarabine: 30 mg/m²/day should be administered as per MSKCC guidelines in the morning over approximately 30-60 minutes on days -6 to -2 (5 doses). Fludarabine dose should be calculated based upon adjusted body weight if the patient is ≥ 125% ideal body weight.
- Cyclophosphamide: 50 mg/kg IV will be given for one dose. It should be administered as per MSKCC guidelines on day -5 after the fludarabine is complete. Cyclophosphamide dose should be adjusted if patient is ≥ 125% ideal body weight (IBW) and should be calculated on adjusted body weight per MSKCC standard of care guidelines. Fluids should be per MSKCC standard of care with diuretics as required to maintain fluid balance.
- Thiotepa: 5 mg/kg/day IV given as a 4 hour infusion on day -4. Thiotepa dose will be calculated based upon adjusted body weight if the patient is ≥ 125% ideal body weight.
- Total Body Irradiation: 200 cGy per dose on days -2 and -1 (2 doses).

9.3 GVHD Prophylaxis

All patients will receive GVHD prophylaxis with 2 drugs as follows:

Cyclosporine

- Cyclosporine A (CSA) will be given per current MSKCC guidelines.
- It will begin on day -3 intravenously in the AM to achieve therapeutic levels per MSKCC guidelines.
- Initial dosing will be per the MSKCC guidelines of 3 mg/kg per dose (adjusted weight in the setting of marked obesity) starting day -3 and dose adjustments should be made on the basis of toxicity and sub- or supra-therapeutic CSA levels.
- Once the patient can tolerate oral medications, CSA can be converted to an oral form.

- In case of major CSA toxicity (e.g. CNS neurotoxicity documented by MRI), CSA should be discontinued. Patients may be re-challenged when clinically appropriate and alternative immune suppression should be substituted per MSKCC guidelines.
- Patients unable to tolerate CSA due to renal impairment should also be considered for an alternative immunosuppressant in addition to mycophenolate mofetil as per MSKCC guidelines.
- Standard patients will receive CSA for approximately 5-6 months in the absence of ongoing GVHD requiring systemic immune suppression. If no history or evidence of GVHD, CSA may be tapered with monitoring for GVHD with the aim to be off immunosuppression by approximately 8-9 months after transplant.
- In patients intolerant of CSA due to renal impairment or other toxicity CSA can be tapered before MMF. This is a reverse taper (see guidelines).
- For patients with GVHD, CSA may be continued for longer time periods according to standard of care guidelines
- If disease progression or persistence occurs, or the patient is considered to be at very high risk of relapse, early taper or cessation of CSA can be considered with close observation for GVHD.

Mycophenolate mofetil (MMF)

- Mycophenolate mofetil (MMF) will be given per current MSKCC MMF guidelines. It should begin on day -3 intravenously in the AM. Standard dose for adults is 15 mg per kg per dose IV q8 hours-see detailed dosing instructions in the
- Obtain therapeutic trough levels as per MSKCC guidelines.
- In preparation for discharge, switch to oral route (CellCept or generic mycophenolate mofetil). For oral conversion round to tablet size. If possible ensure both tablet strengths are given to patient to permit easy taper in clinic. On Pediatric Service can use liquid suspension. Avoid suspension on Adult Service.
- No dose adjustments for renal or liver disease are needed routinely unless severe organ dysfunction.
- If patient is $\geq$ +28 days and without neutrophil engraftment, consideration can be made to dose reduce dosing after discussion with PI or co-PI.
- If no evidence of GVHD, MMF can be tapered at approximately 60-100 days post-transplant. Taper at approximately 10-20% decrements. The aim to be off the drug by approximately 5-6 months. Earlier tapers can be considered if myelosuppression or high relapse risk with very close monitoring for GVHD. Abrupt reductions or cessation should be avoided due to GVHD risk.
- Patients who are intolerant of MMF due to myelosuppression may require earlier taper at the treating physician"s discretion. Do not abruptly stop the drug unless life-threatening toxicity is suspected.
- If the patient is intolerant of CSA, MMF taper may be delayed i.e. do a reverse taper where CSA tapered first (see above).
- If the patient has acute GVHD requiring systemic therapy, MMF should only be tapered if control of GVHD has been obtained

9.4 CB Thaw and Administration

- CB grafts will be received at the MSKCC Cytotherapy Laboratory prior to the start of the preparative regimen.
- Units will be thawed by and released from the Cytotherapy Laboratory according to current standards of practice and release criteria. As per standard practice, ABO blood group, total

nucleated cells (TNC), CD34+ and CD3+ cell number and viability, and sterility will be measured post-thaw.

- A small number of cells (≤ 0.5% of the post-thaw TNC of each unit) may be used for laboratory research studies.
- A sample of each unit should be sent to the American Red Cross for chimerism studies.
- Units should be administered upon arrival to the patient care unit by IV infusion by the nursing staff under supervision of a BMT attending physician. CB infusion nursing guidelines should be followed.
- Pre-medication as per current MSKCC guidelines should be given as appropriate for patient age.
- IV hydration per standard of care. .
- Sample from each CB unit must be submitted to the Diagnostic Molecular Pathology Laboratory.

9.5 Haplo-identical PBSC Collection, Processing and Infusion

- Beginning 5-6 days before the day of transplant, the haplo-identical donor will receive 10 mcg/kg of G-CSF administered subcutaneously daily for at least 5 days as per MSKCC standards of care.
- On the 5th (and 6th if needed) days of G-CSF, the donor will undergo leukapheresis. Leukapheresis will be performed on a continuous flow cell separator according to institutional standards. Mononuclear cell fractions will be pooled. Isolation of CD34+ cells will be performed with the CliniMACS TM System, Miltenyi Biotec.
- **Pheresis will aim for a collection target of at least 6 x 10^6 $CD34^+$ cells/kg recipient body weight** if possible following one or two leukapheresis procedures. The collection target of 6 million will permit the target of 5 million CD34+ cells to be reached post-processing. There is no cap on CD34+ cell dose (see below).
- Bone marrow harvest will not be performed.
- The apheresis product will be first co-incubated with the CliniMacs CD34 reagent (antibody-coated paramagnetic particles). After magnetic labeling and washing, the cells are passed through a high-gradient magnetic separation column in the CliniMACS clinical cell selection device. Magnetically labeled CD34+ cells are retained in the magnetized column, and CD34- cells flow through as the effluent and are discarded. The CD34+ cells retained in the column are eluted by removing the magnetic field and washing the cells through the column. The final CD34+ cell enriched product is concentrated by centrifugation and tested before final release.
- Before infusion, the CD34+ cells will be washed in normal saline for intravenous infusion containing 1% human serum albumin and suspended in a small but unrestricted volume per MSKCC standards.
- QC testing is TNC count, flow cytometry phenotypic analyses of CD45, CD34, and CD3, and viability by 7-AAD, gram stain, and culture.
- The haplo-identical CD34+ cell product can be collected in advance of day 0 if this is required for scheduling purposes and cryopreserved. The collection should occur after the patient has been admitted to guard against collection of haplo-identical cells for a patient whose transplant is subsequently cancelled.
- On day 0 or +1, patients will receive an infusion of haplo-identical CD34+ cell-enriched, T-cell depleted G-CSF-mobilized PBSC.

- **The target of CD34+ cells to be infused as the haplo-identical graft is approximately 5 x 10^6/kg. Higher doses can be given only if the cap on CD3+ cells is met.**
- **To guard against permanent engraftment of the haplo-identical graft, the CD3+ dose will be capped at a maximum of 8 x 10^3/kg.**
- Due to the stringent T-cell depletion, no significant GVHD from the haplo-identical cells is anticipated.
- The CD34+ T-cell depleted PBSC will be infused intravenously over approximately 3-15 minutes per standard of care with close monitoring of vital signs. The patient will be pre-medicated as for blood product transfusions.
- The following details will be collected about the haplo-donor: age, relationship to patient, gender, weight, 10 allele HLA-match, ABO blood group, CMV serostatus, infused CD34+ dose, infused CD3+ dose, day/date of infusion, and whether the product was administered fresh or cryopreserved/ thawed.

9.6 Growth Factor (Filgrastim or G-CSF)

G-CSF 5 mcg/kg once-twice daily IV or once daily SQ (dose rounded to vial size to a maximum of 480 mcg) will be given to all transplant recipients from day +7 until ANC recovery.

10.1 EVALUATION DURING TREATMENT/INTERVENTION

10.2 Prophylaxis Against Infection

Patients will be treated according to the allogeneic BMT standard of care guidelines and will be given prophylaxis against 1) Pneumocystis, 2) Herpes simplex and Herpes Zoster, and 3) fungal infections. Sero-positive patients will be closely monitored for activation of CMV and CMV viremia will be treated according to the BMT guidelines.

10.3 Transfusions

Following initiation of the pre-transplant cytoreduction, all blood products for transfusion (with the exception of the stem cell graft) will be irradiated as per MSKCC guidelines.

10.4 Prophylaxis Against Menstrual Bleeding

Post-pubertal females will be considered for hormonal therapy to suppress menses unless a specific contra-indication to estrogen exists.

10.5 Nutritional Support

Physicians will monitor nutritional status, and high-calorie supplementation and vitamin supplements will be administered as clinically indicated per standard practice.

10.6 Correlative Studies

Laboratory studies will be performed on the **CB graft** using a maximum of 0.5% of each unit on the day of thaw investigating factors associated with unit dominance in patient engraftment. The correlative studies will include:

- Standard characterization of each unit by flow cytometry (Clinical Laboratory, Supervisor Katherine Smith) including analysis of CD34+ and T cells (standard of care and billable). This can be performed on < 5 million mononuclear cells.
- Analysis of the Killer Ig-like receptor (KIR) genotype to assess if alloreactive NK cells are involved in unit predominance (Dr Katherine Hsu"s laboratory).

As a maximum of 0.5% of each unit will be used for research, these studies may be limited by the number of cells available. Studies will be prioritized by the Principle Investigator according to the cell number available.

Laboratory research studies will be performed on the **patient blood post-transplant**.

- To test if NK alloreactivity drives proliferation supporting haplo-PBSC engraftment we will specifically document NK expansion in vivo by following the phenotypic and functional NK repertoires at serial time-points post-transplant, and compare these to the phenotypic and functional repertoire of the donor and patient pre-transplant. Expansion of NK populations can be followed by 12-14 color flow cytometry. Activation markers include intracellular IFN-γ and CD107 (LMP-1, a marker of cytotoxic degranulation).

- To test the recovery of the immune system.

10.7 Post-transplant Evaluation

Post-transplant evaluations are summarized in the following table. Scheduled evaluations for day 21 should be performed +/-2 days, for day 28 should be performed +4/-2 days. For day 60, evaluations should be performed +/-7 days. For day 100, evaluations should be performed +/- 10 days. For 6 and 9 months should be done +/-14 days, and 1 year, 18 months, 2 years should be performed +/-30 days of the targeted date. Immune studies will be done per MSKCC standard of care. Evaluations may be with-held at the treating physicians discretion (eg if the patient has relapsed or is critically ill). Also, additional tests will be performed as clinically indicated.

ACTIVITY	DAY 0 TO CB ENGRAFTMENT	ENGRAFTMENT TO DAY +100	LONG TERM FOLLOW-UP
History & physical		1-2 weekly	Month 6, 9, 12, 24
Chemistry	Per standard of care		Month 6, 9, 12, 24
Counts & differential			Month 6, 9, 12, 24
BM studies: morphology, cellularity, & chimerism	Day 21 if indicated* (Aspirate & core)	Day 100 (Aspirate only unless core clinically indicated)*	Month 6, 12 if needed for clinical management
Chimerism: whole blood (DMP Lab)	Day 14, (21 if no BM aspirate done)	Day 60 & 100	Month 6, 12, 24
Chimerism: blood- (ARC Lab)	Day 28	Day 100	Month 12
GVHD evaluation	-	Once or bi-weekly	Month 6, 12, 24

Immune recovery**(per MSKCC standard)	Day 28	Day 60	Month 3-4, 6, 9, 12, 18, 24
Disease evaluation as appropriate	-	Day 100	Month 6, 12
Research Labs			
NK cell recovery	Day 28	Day 60 & 100	Month 6, 12

* If day 21 BM is not possible or the patient can be adequately assessed from the peripheral blood, peripheral blood chimerism should be performed as a substitute. Also, if BM studies are done prior to day 100 for clinical purposes and it is thought that a repeat is not clinically indicated then day 100 studies can be done on blood only.

** Immune function and NK cell recovery testing can be withheld if the patient has very low circulating white blood cells making testing impossible. Also, if immune recovery studies are done for clinical care purposes and it is thought that a repeat is not clinically indicated they can be withheld.

During the first 100 days patients will be closely monitored as per standard of care. Acute GVHD will be assessed and graded according to MSKCC guidelines. To determine acute GVHD grading, clinical data will be collected weekly or bi-weekly for the first 100 days.

Research blood (1-4 tablespoons/ week) will be obtained to assess the recovery of NK cells as indicated in the Table.

10.8 Evaluation > 100 Days Post-transplant

These should include: history and physical examinations, blood counts and chemistries including liver function tests at a minimum of approximately every 6-8 weeks until 6 months, then at a minimum of approximately every three months for one year, and at approximately 3-6 month intervals until 2 years post transplant and then at least annually as clinically indicated. The patient"s referring physician, in consultation with the MSKCC transplant physician, may assist with follow-up.

Chronic GVHD will be diagnosed and graded according to MSKCC criteria. Assessments will be obtained at approximately day 100, 6, 12 and 24 months after transplant and at additional time points as clinically indicated. Patients who develop chronic GVHD will be treated according to the current standards of care.

Bone marrow aspirate with analysis for chimerism and disease status should be performed at 6 months and one year post transplant if clinically indicated. Blood chimerism should be done at 6, 12, and 24 months. Lymphoid immunophenotyping per MSKCC standards for immune recovery assessment will be evaluated as per MSKCC BMT standard of care guidelines. Patients will be vaccinated as per MSKCC guidelines. Also, research blood (approximately 20 cc) will be obtained to assess the recovery of NK on days 180 and 1 year.

11.1 TOXICITIES/SIDE EFFECTS

11.2 Toxicity Grading

Toxicities will be graded according to Adult BMT Guidelines.

11.3 Total Body Irradiation (TBI)

Nausea and vomiting: most patients will experience nausea and vomiting after irradiation which can be significantly diminished with anti-emetics. Parotitis: some patients may experience symptomatic parotitis within the first 24 hours post radiation. This resolves spontaneously over several days. Diarrhea: most patients develop some diarrhea in the first week post irradiation which is treated symptomatically. Fever: low grade fever may occur for 24 hr post irradiation. This is treated symptomatically. Skin changes: Erythema may occur in patients within 24 hours and resolves in 2-3 days. Most patients will get some degree of hyperpigmentation within 2-3 weeks. Alopecia is always seen but is usually reversible. Myelosuppression and immune suppression is a major toxicity and is treated by donor stem cell infusion and supportive care. Mucositis: most patients will develop moderate to severe mucositis of the oral and GI tracts which will be managed with aggressive supportive care. High doses of radiation in combination with high dose chemotherapy may contribute to damage to vital organs such as the lung or the liver that is sometimes associated with myeloablative conditioning but is not attributable to one specific agent. Late effects include cataracts, sterility, growth retardation, second malignancies and hypothyroidism. Cataract formation is seen in < 30% of patients treated with hyperfractionated TBI. Sterility is extremely common following total body irradiation and administration of alkylating chemotherapy with the risk increasing with the number of years since puberty. Hypothyroidism will be routinely monitored post transplant and treated with hormonal replacement as indicated.

11.4 Cyclophosphamide

Nausea and vomiting: virtually all patients will experience nausea and vomiting after high dose cyclophosphamide. This can be significantly diminished with anti-emetics. Diarrhea: most patients develop some diarrhea in the first week post cyclophosphamide. This is treated symptomatically. Myelosuppression and immune suppression is a major toxicity and is treated by donor stem cell infusion and supportive care. Mucositis: most patients will develop moderate to severe mucositis of the oral and GI tracts which will be managed with aggressive supportive care. Skin changes: Transient skin rashes have been described. Alopecia is always seen but is usually reversible. Hemorrhagic cystitis is a potential complication, can be variable in severity and occasionally can be severe. The risk of cystitis will be reduced by aggressive supportive care. Fluid weight gain and edema is associated with this fluid flush but is transient and can be treated with diuretics if necessary. A syndrome of inappropriate anti-diuretic hormone (SIADH) causing hyponatremia can be seen but is transient and will spontaneously resolve after drug administration. Cardiomyopathy has been described with cyclophosphamide but is very rare. High doses of cyclophosphamide in combination with high dose radiation may contribute to damage of vital organs such as the lung or the liver. This is sometimes associated with the myeloablative conditioning as used in this study but is not attributable to one specific agent. Late effects include sterility and the possibilities of second cancers.

11.5 Fludarabine

Fludarabine may contribute to nausea, vomiting, mouth sores, and diarrhea which are primarily due to the high dose cyclophosphamide and TBI. Jaundice and elevations of liver enzymes have also been described. Transient skin rashes have been described. Myelosuppression and immune suppression is a major toxicity and is treated by donor stem cell infusion and supportive care. Effects on the nervous system are not expected at the doses used in this protocol, but if they occur could include confusion, coma, weakness or numbness, loss of balance, difficulty walking, or loss of vision and could be very serious or lethal.

11.6 Thiotepa

Side effects of thiotepa include: alopecia, nausea, vomiting, and diarrhea. Thiotepa can also cause myelosuppression, pancytopenia, sterility and fevers. Other less likely side effects include dizziness and transient hepatic transaminase elevation. Rare but serious side effects include CNS toxicity manifested by headache, mild cognitive dysfunction, disorientation, confusion, irritability, and bizarre behavior; as well as, interstitial pneumonitis and renal failure.

11.7 Mycophenolate Mofetil (MMF)

The major toxicity of MMF is immune suppression which leads to increased risk for infection. This is managed with aggressive supportive care with both prophylaxis and treatment of infectious complications. Other potential side-effects include myelosuppression, headache, insomnia, aches and pains, rash, nausea, anorexia and diarrhea. There is also a very rare side effect known as progressive multifocal leukoencephalopathy (PML), which is a progressive disease of the nervous system that can cause severe disability or death. A very small number of cases of PML have been reported in patients treated with MMF. PML can cause hemiparesis, confusion, cognitive deficiencies and ataxia.

11.8 Cyclosporine-A (CSA)

The major toxicity of CSA is immune suppression which leads to increased risk for infection. This is managed with aggressive supportive care with both prophylaxis and treatment of infectious complications. Renal dysfunction is common and is treated with good hydration and reduction of the dose if necessary. Electrolyte abnormalities involving potassium and magnesium are also common and electrolytes must be closely monitored. Increased blood pressure is common and is treated with anti-hypertensive medication(s). Neurological side effects include tremor (common), seizures (rare), confusion, ataxia, cortical blindness (rare), and peripheral neuropathies and are usually reversible with cessation of the medication. While mild to moderate microangiopathic hemolysis is relatively common, serious thrombotic thrombocytopenic purpura (TTP) is rarely seen. Gastrointestinal side-effects include anorexia, nausea, swollen gums, and hyperbilirubinemia. Skin changes include hirsutism and gingival hyperplasia.

11.9 G-CSF (Neupogen)

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

11.10 Mesna (Mesnex®)

Mesna has little systemic side effects; side effects that are observed are usually due to the concomitant chemotherapy. Mesna is an uroprotectant and is used with the high dose cyclophosphamide.

11.11 Blood Product, CB and PBSC Infusions

Infusions of blood products may produce volume overload which can be managed with diuretics. They may also induce allergic reactions of variable severity, many of which can be prevented or mitigated by pre-medication with antipyretics, and antihistamines. Any donor or blood product may also transmit serious infections (e.g., CMV, hepatitis, HIV). To circumvent this, stem cell and blood donors are screened according to AABB and FACT guidelines. CMV

antibody negative blood products will be used in CMV seronegative individuals whenever possible. All blood products (other than the stem cell graft) are irradiated to circumvent the risk of GVHD caused by contaminating lymphocytes.

Toxicities potentially associated with the infusion of the CB graft include DMSO toxicity and side effects from red cells and may include changes in heart rate or rhythm, changes in blood pressure, fever, chills, sweats, nausea/vomiting, diarrhea, abdominal cramping, headache, dyspnea, presence of DMSO taste and odor, hemoglobinuria, and acute renal failure. However due to the dilution, hydration, and pre-medication these toxicities are unlikely. The process of CB engraftment can also be associated with a pre-engraftment syndrome characterized by fever and manifestations of capillary leak syndrome. This process is highly responsive to corticosteroid therapy and this will be treated according to accepted clinical practice.

The definitions and reporting of serious adverse events (SAEs) is defined in Section 17.2.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Clinical Endpoints The primary aim of this study is to obtain an estimate of the speed of neutrophil recovery after double-unit CBT with a T-cell depleted haplo-identical allograft. Overall survival and disease free survival at one and two years will be monitored as secondary endpoints.

12.1. Neutrophil and Platelet Engraftment and Graft Failure (GF)

Patients will be monitored for donor cell engraftment as evidenced by neutrophil and platelet recovery and donor chimerism in the marrow and blood at serial time-points post-transplant.

The day of **neutrophil recovery** is defined as the first of 3 consecutive days in which the absolute neutrophil count (ANC) is $\geq$ 0.5. The cumulative incidence of neutrophil recovery will be reported at day 45 post-transplant. **Sustained neutrophil engraftment** is neutrophil engraftment without secondary graft failure.

The day of **platelet recovery** is defined as the first of 3 consecutive days in which platelet counts are $\geq$ 20,000 without requiring platelet transfusions in the previous 7 days. The cumulative incidence of platelet recovery will be reported at day 180.

Engraftment of each CB unit and the haplo-identical donor will be defined tracked at serial time-points.

Primary graft failure is diagnosed when the patient fails to achieve an ANC $\geq$ 0.5 within the first 45 days post-transplant. Infusion of another source of stem cells as a treatment of graft failure prior to day 45 will also be considered primary graft failure. Patients who die before day 45 without count recovery will be classed as graft failures after day 28 and as not evaluable if death occurs without count recovery between days 0-28. Should evaluation of primary graft failure indicate that the patient's leukemia has persisted or recurred the patient will be classified as having progressive disease or relapse and not as having primary graft failure.

Secondary graft failure is defined as donor-derived neutrophil engraftment followed by severe neutropenia (ANC < 0.5 for more than 7 consecutive days unresponsive to growth factors in the absence of relapse), or an absence of donor cells in the marrow and/or blood without leukemic relapse. Patients with suspected graft failure will be evaluated with bone marrow biopsy to assess hematopoiesis and to test for residual or recurrent leukemia. Patients who suffer graft failure will be managed per MSKCC BMT guidelines.

12.2. Graft-Versus-Host Disease (GVHD)

Acute GVHD is manifested by skin rash, nausea, vomiting, diarrhea and ulceration of the intestines, hyperbilirubinemia and hepatitis, and suppressed or delayed recovery of the hematopoietic and immune system. Standard clinical criteria, and histological grading of skin, liver or gastrointestinal pathology where possible, will be used to establish and grade acute GVHD. In the first 100 days after transplant patients will be assessed by a transplant physician for the development of acute GVHD approximately weekly. Data will be collected as per standard practice of the Adult BMT service. Patients with moderate to severe acute GVHD (grade II-IV) will be treated as per standard of care. Patients failing to respond to steroids will be considered for treatment with standard or experimental immunosuppressive agents Chronic GVHD is characterized to varying degrees by sclerosis of lacrimal and salivary ducts, scleroderma-like changes of the skin, chronic inflammation and scarring of the gastrointestinal tract with consequent malabsorption and diarrhea, inflammation of the liver, suppression of the immune system and occasionally other auto-immune phenomena (eg auto-immune hemolysis) or involvement of other organs (eg pulmonary involvement). Chronic GVHD will be diagnosed and graded according to the MSK criteria and treated with standard or experimental immunosuppressive therapy. Patients will be assessed for GVHD at day 100, 6 months, 1, and 2 years.

12.3. Transplant-related Mortality (TRM)

TRM is defined as death at any time from the commencement of pre-transplant conditioning due to any cause other than malignant relapse (with the exception of accidents such as automobile accidents). Stopping rules are in place to consider cessation of the study if TRM at day 100 is excessive (see Section 14).

12.4. Relapse

Relapse of malignancy is a secondary endpoint of this study and will be defined by accepted clinical practice eg an increasing number of blasts/malignant cells of recipient origin in the marrow over 5%, by the presence of circulating peripheral blasts, or by the presence of malignant cells in any extramedullary site. Cytogenetic (eg if a diagnosis of CML) or flow cytometric analysis or molecular studies of the marrow and/or peripheral blood may also be obtained for the diagnosis of relapse. Isolated molecular persistence or reappearance of bcr-abl without cytogenetic positivity will not be considered relapse.

12.5 Immunologic Recovery

Immunophenotyping of T, B, and NK cells, and T-cell proliferations in response to non-specific mitogens will be performed at serial time points after transplant to measure immune recovery as outlined in section 10.6. Patients may be re-immunized post-transplant according to the MSKCC guidelines. Immune recovery data will be analyzed at completion of study and interim analysis of immune recovery will be permitted.

13.0 CRITERIA FOR REMOVAL FROM STUDY

Patients may be removed from the study at any point deemed appropriate by the Principal Investigator or if requested by the patient. However, once the pre-transplant conditioning regimen is given, patients will continue on study until after administration of their stem cells and will receive supportive care as appropriate. Failure to rescue the patient with stem cells after cytoreduction would most likely be fatal.

14.0 BIOSTATISTICS

Clinical Endpoints

A phase 2 study design will be utilized to obtain preliminary estimates of clinical efficacy and safety of a myeloablative cord blood transplant combined with a T-cell depleted haplo-identical allograft. The population under study is pediatric and adult patients with high-risk or advanced hematologic malignancies. At the time of transplant patients will be classified into standard (Arm A) or high (Arm B) risk groups (see section 6). A maximum of 26 evaluable patients will be accrued into Arm A (standard risk for TRM) and 75 into Arm B (high risk for TRM). The accrual period will be approximately 3-4 years with an additional two years of follow up after accrual is completed. All endpoints in this study will be assessed separately in the two risk groups.

The primary endpoint of the study is the number of days to neutrophil recovery. Historically, the median time to neutrophil recovery for myeloablative double unit cord transplant patients is 25 days with all engrafting patients recovering neutrophils by day 45. A design that differentiates between population median recovery times of 25 days and 15 days will be used to assess the neutrophil recovery rate in this trial. A truncated exponential maximum likelihood test will be used to test this hypothesis. For the purpose of the power calculation, it is assumed that the neutrophil recovery times are exponentially distributed and for the anticipated 5% of the patients that do not recover, the recorded time will be day 46. This design has power equal to 0.90 if the population median neutrophil recovery time for patients treated under the proposed approach is 15 days; the one-sided test size is 0.10 when the median recovery time is 25 days.

In addition, the time to platelet recovery will also be tested relative to MSKCC historical controls. Based on prior data, the null hypothesis is that the median time to platelet recovery is 48 days. The 26 patient study has power equal to 0.90 if the population median time to platelet recovery is 29 days in the current study. Patients that do not have platelet recovery will be scored as day 180. A one-sided 0.10 size test will be used to determine if platelet recovery is improved relative to the historical data.

After the initial 18 high risk (Arm B) patients were treated on the protocol with a haplo-identical peripheral blood stem cell graft of 1.0 - 3.0 x 10^6 CD34+ cells/kg, the haplo-identical CD34+ cell dose was increased to 5.0 x 10^6/kg due to promising results in the initial experience with 3 million CD34+ cells. The initial 26 patient high risk cohort was completed and the primary endpoint of ANC recovery was met. To further ascertain the clinical efficacy of the addition of a higher dose of haplo-identical CD34+ cells, the number of patients studied will be increased. An additional 49 patients will be added to Arm B as a high risk patient extension cohort for a total of 75 patients. The Arm B extension cohort will utilitize the same efficacy evaluations as the primary cohort. The stopping rules below for the high risk Arm B have been amended to include the primary and extension cohorts.

The study design includes early termination in the event of excessive graft failure, severe acute GVHD, or early TRM/ death from relapse within the first 100 days post-transplant. The power calculations used to assess the adequacy of the treatments with respect to these endpoints are given as guidelines in the tables below. In the event that the stopping boundary is crossed in one risk group, the study will continue accrual in the other risk group. The stopping rules for the High Risk (Arm B) cohort includes the primary 26 patients cohort and the additional 49 patient extension cohort.

Stopping Rule Guidelines for Arm A (Standard Risk) Group

Failure Type	Failure Boundary to Stop Study	Projected Probability of Failure	Probability of Boundary Crossing
Graft failure	4 within first 11 patients 5 within first 16 patients 6 within first 22 patients 7 within 26 patients	0.13	0.09
		0.35	0.90
Grade IV Acute GVHD	3 in the first 14 patients 4 in the first 23 patients 5 within 26 patients	0.07	0.10
		0.25	0.89
TRM/ Death from Relapse Within 100 Days	3 in the first 10 patients 4 in the first 16 patients 5 in the first 23 patients 6 within 26 patients	0.09	0.09
		0.30	0.91

Stopping Rule Guidelines for Arm B (High Risk) Group and Arm B Extension Cohort

Failure Type	Failure Boundary to Stop Study	Projected Probability of Failure	Probability of Boundary Crossing
Graft failure	5 in the first 11 6 in the first 16 7 in the first 26 9 in the first 32 10 in the first 38 11 in the first 43	0.13	0.1
	12 in the first 54 13 in the first 60 14 in the first 66 15 in the first 72 16 at any point	0.35	0.99
Grade IV Acute GVHD	5 in the first 11 6 in the first 14 7 in the first 18 8 in the first 22 9 in the first 27 10 in the first 31 11 in the first 35	0.17	0.1
	12 in the first 39 13 in the first 44 14 in the first 48 15 in the first 53 16 in the first 58 17 in the first 62 18 in the first 67 19 in the first 72	0.4	0.99

	20 at any point		
TRM/ Death from Relapse Within 100 Days	7 in the first 13 8 in the first 16 9 in the first 19 10 in the first 21 11 in the first 24 12 in the first 27 13 in the first 31 14 in the first 34 15 in the first 37 16 in the first 40 17 in the first 43	0.25	0.07
	18 in the first 46 19 in the first 50 20 in the first 53 21 in the first 56 22 in the first 59 23 in the first 63 24 in the first 66 25 in the first 69 26 in the first 73 27 at any point	0.5	0.98

Each patient will followed for at least two years and subsequently as clinically indicated. If the study is not terminated early due to graft failure, grade IV GVHD, or TRM/early death, the probability of remaining alive for one year will be assessed. Arm A includes patients with high risk hematologic malignancies but with standard TRM risk for CBT. Arm B includes high risk patients not usually offered transplant or those considered at very high risk for TRM.

For the standard risk group (Arm A), a single stage design that differentiates between population 1 year overall survival rates of 0.60 and 0.85 will be used determine treatment activity. The treatment will be considered insufficiently active if the probability of remaining alive for 1 year in this patient population is less than or equal to 0.60. If at least 20 of the 26 patients remain alive for one year, the treatment will be considered successful. This design has power greater than 0.90 if the probability of overall survival at one year is 0.85, using a 0.10 one-sided size test. The high risk group (Arm B) has a larger sample size and survival will be analyzed according to disease risk and patient comorbidity scores.,

The use of a one-stage design is due to the one-year follow-up time for this endpoint. For each group, the Kaplan-Meier estimates of overall and disease-free survival will be computed over time. In addition to the survival analyses, the cumulative incidence of neutrophil and platelet engraftment, grade 2-4 acute GvHD, chronic GvHD, TRM, relapse, and length of hospitalization will be computed. Kernel smoothing will be used to assess the level of chimerism, neutrophil count, platelet count, NK, T cell and B cell populations over time. The outcomes of this study will also be compared to MSKCC double-unit CBT historical controls.

15.1 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.2 Research Participant Registration

Research Participant Registration

Confirm eligibility as defined in the section entitled Inclusion/Exclusion Criteria. Obtain informed consent, by following procedures defined in section entitled Informed Consent Procedures. During the registration process registering individuals will be required to complete a protocol specific Eligibility Checklist. The individual signing the Eligibility Checklist is confirming whether or not the participant is eligible to enroll in the study. Study staff are responsible for ensuring that all institutional requirements necessary to enroll a participant to the study have been completed. See related Clinical Research Policy and Procedure #401 (Protocol Participant Registration).

15.3 Randomization

Not applicable.

16.1 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study at MSKCC. The responsibilities of the RSA include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordination of the activities of the protocol study team.

The data collected for this study will be entered into a secured database Clinical Research Database (CRDB) and a database in a secured Adult BMT drive. Source documentation will be available to support the computerized patient record.

16.2 Quality Assurance

Weekly registration reports will be generated to monitor patient accruals and completeness of registration data. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates and extent and accuracy of evaluations and follow-up will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

Random-sample data quality and protocol compliance audits will be conducted by the study team, at a minimum of two times per year, more frequently if indicated.

16.3 Data and Safety Monitoring

The Data and Safety Monitoring Plans (DSM) at Memorial Sloan-Kettering cancer Center were approved by the National Cancer Institute in September 2001. The plans address the new policies set forth by the NCI in the document entitled “Policy of the National Cancer Institute for Data and Safety Monitoring of Clinical Trials” which can be found at: Http://cancertrials.nci.nih.gov/researchers/dsm/index.html. The DSM Plans at MSKCC were established and are monitored by the Clinical Research Administration The MSKCC Data and Safety Monitoring Plans can be found on the MSKCC Intranet at: http://mskweb2.mskcc.org/irb/index.htm.

There are several different mechanisms by which clinical trials are monitored for data, safety and quality. There are institutional processes in place for quality assurance (e.g., protocol monitoring, compliance and data verification audits, therapeutic response, and staff

education on clinical research QA) and departmental procedures for quality control, plus there are two institutional committees that are responsible for monitoring the activities of our clinical trials programs. The committees: *Data and Safety Monitoring Committee (DSMC)* for Phase I and II clinical trials, and the *Data and Safety Monitoring Board (DSMB)* for Phase III clinical trials, report to the Center"s Research Council and Institutional Review Board.

During the protocol development and review process, each protocol will be assessed for its level of risk and degree of monitoring required. Every type of protocol (e.g., NIH sponsored, in-house sponsored, industrial sponsored, NCI cooperative group, etc.) will be addressed, and the monitoring procedures will be established at the time of protocol activation.

17.1 PROTECTION OF HUMAN SUBJECTS

17.2 Privacy

MSKCC"s Privacy Office may allow the use and disclosure of protected health information pursuant to a completed and signed Research Authorization form. The use and disclosure of protected health information will be limited to the individuals described in the Research Authorization form. A Research Authorization form must be completed by the Principal Investigator and approved by the IRB and Privacy Board (IRB/PB).

17.3 Serious Adverse Event (SAE) Reporting

An adverse event is considered serious if it results in ANY of the following outcomes:

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition

Note: Hospital admission for a planned procedure/disease treatment is not considered an SAE.

SAE reporting is required as soon as the participant signs consent. SAE reporting is required for 30-days after the participant"s last investigational treatment or intervention. Any events that occur after the 30-day period and that are at least possibly related to protocol treatment must be reported.

If an SAE requires submission to the IRB office per IRB SOP RR-408 „Reporting of Serious Adverse Events", the SAE report must be sent to the IRB within 5 calendar days of the event. The IRB requires a Clinical Research Database (CRDB) SAE report be submitted electronically to the SAE Office as follows:

For IND/IDE trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to saemskind@mskcc.org.

For all other trials: Reports that include a Grade 5 SAE should be sent to saegrade5@mskcc.org. All other reports should be sent to sae@mskcc.org.

The report should contain the following information:

Fields populated from CRDB:

- Subject‟s initials
- Medical record number
- Disease/histology (if applicable)
- Protocol number and title

Data needing to be entered:

- The date the adverse event occurred
- The adverse event
- The grade of the event
- Relationship of the adverse event to the treatment (drug, device, or intervention)
- If the AE was expected
- The severity of the AE
- The intervention
- Detailed text that includes the following
    - A explanation of how the AE was handled
    - A description of the subject‟s condition
    - Indication if the subject remains on the study
- If an amendment will need to be made to the protocol and/or consent form
- If the SAE is an Unanticipated Problem

The PI‟s signature and the date it was signed are required on the completed report.

For IND/IDE protocols:
The CRDB SAE report should be completed as per above instructions. If appropriate, the report will be forwarded to the FDA by the SAE staff through the IND Office.
NOTE: potentially serious toxicities are an expected part of transplant therapy. The reportable serious adverse events (SAEs) will be based on the most recent version of the Adult and Pediatric BMT Adverse Event Reporting Standard Operating Procedures.

17.2.1 Costs

The patient will be responsible for the costs of medical care, including the UCB and haplo-identical graft, all hospitalizations and any transplant complications. Research tests will be done at no cost to the patient.

18.1 INFORMED CONSENT PROCEDURES

Before protocol-specified procedures are carried out, consenting professionals will explain full details of the protocol and study procedures as well as the risks involved to participants prior to their inclusion in the study. Participants will also be informed that they are free to withdraw from the study at any time. All participants must sign an IRB/PB-approved consent form indicating their consent to participate. This consent form meets the requirements of the Code of Federal Regulations and the Institutional Review Board/Privacy Board of this Center. The consent form will include the following:

1. The nature and objectives, potential risks and benefits of the intended study.
2. The length of study and the likely follow-up required.
3. Alternatives to the proposed study. (This will include available standard and investigational therapies. In addition, patients will be offered an option of supportive care for therapeutic studies.)
4. The name of the investigator(s) responsible for the protocol.
5. The right of the participant to accept or refuse study interventions/interactions and to withdraw from participation at any time.

Before any protocol-specific procedures can be carried out, the consenting professional will fully explain the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must agree to the Research Authorization component of the informed consent form. Each participant and consenting professional will sign the consent form. The participant must receive a copy of the signed informed consent form.

19.0 REFERENCES

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