A Pilot Trial of GVHD Prophylaxis with Post Transplant Cyclophosphamide for Patients with Renal Insufficiency Undergoing a Conventional 8/8 HLA-matched Related or Unrelated Donor Allogeneic Hematopoietic Stem Cell Transplant

PROTOCOL FACE PAGE FOR
MSKCC THERAPEUTIC/DIAGNOSTIC PROTOCOL

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

1.0 PROTOCOL SUMMARY AND/OR SCHEMA

This is a single center, pilot trial evaluating the safety of post transplant cyclophosphamide as GVHD prophylaxis alone following a conventional (unmodified) matched related or unrelated allogeneic hematopoietic stem cell transplant (HSCT) in patients with renal insufficiency. Patients with acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (AML), high risk forms of myelodysplastic syndrome (MDS), chronic myelogenous leukemia (CML) unresponsive or intolerant of TKIs, advanced myeloproliferative disease (MPD) and non Hodgkins lymphoma (NHL) who fulfill eligibility requirements and consent to treatment will receive a reduced intensity (RIC) regimen followed by infusion of a 8/8 matched related or unrelated donor stem cell graft.

The RIC regimen will consist of melphalan plus fludarabine. Patients eligible for this protocol are those who require a conventional graft but are at high risk of renal failure and other complications associated with the use of standard GVHD prophylaxis including calcineurin inhibitors. They will receive post transplant cyclophosphamide (50 mg/kg/day IV) on days +3 and +4. Bone marrow (BM) will be the preferred choice for the graft with peripheral blood stem cells (PBSC) as the secondary choice. If PBSC grafts are used, the CD34+ cell dose will be capped to limit the number of CD3+ cells.

Separately in the BM and PBSC cohorts, the trial design will de-escalate the GVHD prophylaxis to determine whether cyclophosphamide alone can be used. The general design of the trial will follow the 3-by-3 design typically used in phase I dose escalation trials.

As shown in the table below, the BM cohort will first start with sirolimus (Siro) plus cyclophosphamide, which can be de-escalated if excessive grade IV GVHD is not observed. If excessive GVHD is observed at dose level 1 (Siro+ Cyclophosphamide), a dose level -1 will be explored and mycophenolate (MMF) will be added to the regimen.

BM De-escalation

Level	Prophylaxis
-1	MMF + Siro+ Cyclophosphamide
1	Siro+ Cyclophosphamide
2	Cyclophosphamide

The table below describes the de-escalation in the PBSC cohort. If excessive GVHD is observed in dose level 1 (MMF+Siro+ Cyclophosphamide), a dose level -1 will be explored and methylprednisolone will be added to the regimen.

PBSC De-escalation Level	Prophylaxis
-1	Methylprednisolone+ MMF+ Siro+ Cyclophosphamide
1	MMF+ Siro+ Cyclophosphamide
2	Siro+ Cyclophosphamide
3	Cyclophosphamide

This protocol offers a strategy for post transplant cyclophosphamide as primary GVHD prophylaxis and is novel in the capping of the CD34/CD3 dose and employing GVHD prophylaxis that is not renal toxic.

Melphalan 70 mg/m^2 d-6 and -5 Fludarabine 25 mg/m^2 d-6 - -2

2.0 OBJECTIVES AND SCIENTIFIC AIMS

1) To evaluate a safe minimal combination of GVHD prophylaxes for patients with renal insufficiency not appropriate for standard calcineurin inhibitor prophylaxis who receive a bone marrow (BM) or peripheral blood stem cell (PBSC) (with limited CD34 and T cell dosing) grafts.

Secondary objectives

1) To assess overall incidence of acute and chronic GVHD by 2 years
2) To assess 100-day transplant-related mortality
3) To assess the incidence of graft failure.
4) To assess 2-year overall survival (OS) and disease-free survival (DFS)
5) To determine
   a) the incidence acute renal insufficiency, requiring dialysis
   b) the worsening of chronic renal insufficiency
6) To assess infectious complications
7) To evaluate the time to immune reconstitution, defined as PHA >50% LLN and CD4>200

3.0 BACKGROUND AND RATIONALE

Graft-versus-host disease (GVHD) is a major cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT). While the incidence of severe acute GVHD has declined over the past decade, up to 70% of patients continue to develop grade 2-4 acute GVHD following a well matched related or unrelated graft[1]. Long-term immunosuppression is required in 30-40% of patients who develop GVHD[1-6]. In the event of steroid-refractory disease, the mortality rate is in excess of 75%, often due to uncontrollable infectious complications[7].

Pharmacologic GVHD prophylaxis primarily includes the combination of a calcineurin inhibitor with methotrexate (MTX) or MMF[1,8]. Calcineurin inhibitor-based GVHD prophylaxis is associated with a large number of adverse effects, including nephrotoxicity[4,9-12]. The use of calcineurin inhibitors in patients with poor renal reserve is often not feasible and potentially jeopardizes the individual's ability to receive an unmodified graft. Over the past decade, the

incidence of nephrotoxicity within the first 100 days post transplant has decreased dramatically. However 43% of patients continue to develop a 2-3 fold rise in serum creatinine[1]. Though rare, post transplant hemodialysis is required in 5% of patients[1]. Additionally, calcineurin-based prophylaxis offers suboptimal GVHD control. Acute GVHD occurs in 18-40% of patients that receive an unmodified graft followed by conventional calcineurin inhibitor-based prophylaxis[9,10]. The incidence of chronic GVHD among individuals that receive standard pharmacologic prophylaxis ranges from 24-58%[9,10]. Two large phase III studies have compared tacrolimus to cyclosporine in combination with MTX. Both trials showed a lower risk of acute GVHD for the tacrolimus arm, but similar survival[9,10]. None of the currently used calcineurin inhibitor based acute GVHD regimens have had any significant impact in reducing the risk of chronic GVHD. Chronic GVHD has emerged as one of the most common and debilitating complications of allogeneic SCT and a major contributor to poor quality of life post SCT[13].

Three calcineurin inhibitor free GVHD strategies have been studied for acute GVHD prevention: 1) T cell depletion of the donor graft[2,6,14-24]; 2) post transplant cyclophosphamide[25-29] and 3) sirolimus containing regimens[30]. Prospective comparisons of these methods are needed to determine potential benefits of one strategy over another as well as comparative effectiveness studies with other acute GVHD prophylaxis regimens in specific patient populations. While MSKCC has expertise in TCD, which can be used in patients with renal insufficiency prior to transplant, the disease in many patients precludes its appropriateness for a potentially curative transplant. Furthermore, although sirolimus can be used for patients with renal insufficiency, it has not demonstrated efficacy as first line therapy alone for acute GVHD prophylaxis in patients requiring a conventional graft. Post transplant cyclophosphamide is associated with relatively low rates of grade III-IV acute GVHD and chronic GVHD[6]. The GVHD control provided by post transplant cyclophosphamide is due to its ability to eliminate proliferating alloreactive T cells and preserve the expansion of CD4+CD25+CD127-FoxP3+ T cells (regulatory T cells or T-regs)[25-28]. Tregs are critically involved in the control of GVHD, through IL-10, TGF-beta, and direct cell-cell mechanisms[31,32]. Conversely, calcineurin-based prophylaxis greatly impairs post transplant Treg expansion by suppressing vital IL-2-dependant signaling[3,5,31,32].

High-dose, post transplant cyclophosphamide is an effective form of GVHD prophylaxis, allowing for graft tolerance without sacrificing GvL. Compared with calcineurin-based prophylaxis, only 5% of patients that receive post transplant cyclophosphamide develop renal toxicity[25]. Preclinical murine transplant studies demonstrated that post transplant cyclophosphamide achieved successful engraftment of MHC-mismatched marrow, allowed for add-back donor lymphocyte infusions when challenged with a leukemia cell line, and controlled GVHD[26,27]. In 2002, GVHD prophylaxis with high-dose post transplant cyclophosphamide was explored clinically in a phase I trial. Thirteen patients received nonmyeloablative conditioning with fludarabine, cyclophosphamide, and TBI[29]. GVHD prophylaxis consisted of post transplant cyclophosphamide 50 mg/kg once on day +4, and mycophenolate mofetil with tacrolimus beginning on day +5[29]. The trial demonstrated that post transplant cyclophosphamide was safe in combination with standard prophylaxis, without an unacceptable level of graft failure or relapse[29].

High-dose post transplant cyclophosphamide was later evaluated in a phase I/II trial of 68 patients with high risk leukemia and lymphoid malignancies receiving grafts from HLA-haploidentical related donors[28]. The preparative regimen again consisted of fludarabine, cyclophosphamide, and TBI[28]. High-dose cyclophosphamide was administered on day +3, or days +3 and +4[28]. MMF and tacrolimus were initiated the following day after completing the infusion of cyclophosphamide[28]. While patients transplanted for lymphoid diseases performed statistically better, the overall and event-free survival was 36% and 26%. The risk of relapse was 51% at 1 year[28].

In 2010, GVHD prophylaxis with post transplant high-dose cyclophosphamide, **without any additional pharmacologic immunosuppression**, was studied in a phase I/II trial of advanced hematologic malignancies receiving BM grafts with a targeted total nucleated cell dose of 4×10^8/kg. [26] This study differed from the previous trials, in that all 117 patients received myeloablative conditioning with busulfan and cyclophosphamide. Overall, the incidence of graft failure was low, with a total of 5 patients developing primary or secondary graft failure. The day +100 cumulative incidence of grade II-IV acute GVHD for all patients was 43%. The cumulative incidence of relapse at 2 years was 44%. The OS at 2 years after BMT was 55%. No significant difference in grade II-IV GVHD, relapse, or OS was observed between the recipients of related or unrelated donor grafts. Additionally, the cumulative incidence of chronic GVHD was 10% at 2 years. The trial demonstrated that post transplant cyclophosphamide alone in this protocol design offered prophylaxis for acute GVHD, limited chronic GVHD, facilitated stem cell engraftment, and was safe in patients with serum creatinine levels up to 2.5 mg/dL.

In the last few years, publications have been predominantly retrospective but have included patients who received some GVHD prophylaxis (mostly including calcineurin inhibitors), limited if any patients with renal insufficiency, and most using bone marrow rather than PBSC grafts. Two have started to limit the number of CD34+ cells in the graft as proposed in this study. [32-34]

Currently, the criteria for renal function employed in most of the transplant protocols is a serum creatinine within the normal range, or if not in the normal range, a creatinine clearance (CrCl) of > 50 or MDRD > 60 ml/min. Generally, lean body mass (which is the source of creatinine) decreases with age. But renal function also declines, resulting in less CrCl. Serum creatinine in the older patients will not increase until 50% of nephrons are no longer functional and thus a normal creatinine is not a reliable indicator of adequate renal function. Because of these age-related changes and those associated with lean body mass, which can change significantly in patients with cancer, any elevation of creatinine is significant. Thus serum creatinine is not a reliable indicator of renal function in the older or debilitated patient, renal function is best assessed and monitored with CrCl or MDRD. For patients with reduced renal function, the preference for transplant at MSKCC has been to offer a TCD transplant which markedly reduces the need for nephrotoxic drugs that can further impact negatively on the renal function and transplant outcome. For patients with persistent disease at the time of transplant, a TCD transplant is not an appropriate option and the general practice is to offer a conventional transplant which requires the use of calcineurin inhibitors for GVHD prophylaxis. The calcineurin inhibitors can result in the development of renal insufficiency or can exacerbate renal insufficiency that is already present. If the patient is unable to receive a calcineurin inhibitor around the time of the transplant there is a high likelihood that they will develop severe GVHD which is often lethal. Thus patients with significant renal insufficiency are usually not considered candidates for conventional transplants and thus may not be eligible for any transplant which could be curative for their disease. The approach described here offers these patients an option.

4.0 OVERVIEW OF STUDY DESIGN/INTERVENTION

4.1 Design

This is a single center, pilot trial evaluating the safety of post transplant cyclophosphamide as GVHD prophylaxis following a matched related or unrelated allogeneic hematopoietic

stem cell transplantation. Patients with ALL, AML, high risk forms of MDS, CML intolerant of or unresponsive to TKIs, advanced MPD, and NHL who fulfill eligibility requirements and consent to treatment will receive a reduced intensity (RIC) conditioning regimen with a 8/8 matched related or unrelated donor stem cell graft per MSKCC protocol. If PBSC are used, the CD34+ cell dose will be capped to limit the number of CD3+ cells. To investigate whether Cyclophosphamide alone can be used as GVHD prophylaxis, both cohorts will receive additional prophylaxis initially which will be removed if excessive grade IV GVHD at day +100 is not observed. The de-escalation schema will follow a 3-by-3 strategy typically used in dose escalation studies. Two days of posttransplant cyclophosphamide will be the main form of GVHD prophylaxis administered on d+3 and d+4 to all patients. Additional GVHD prophylaxis will be given initially until it is evident that the risk of grade IV GVHD is low. If acute grade III-IV GVHD exceeds 33% in any group, the posttransplant prophylaxis in subsequent patients will be increased to that used in the previous cohort. If excessive grade III-IV GVHD occurs in the first cohort of patients receiving PBPC grafts, methylprednisolone 0.5 mg/kg daily will be added to the Siro, MMF and cyclophosphamide, and an additional 3-6 patients who receive PBPC grafts will be treated with that regimen. If excessive grade III-IV GVHD occurs in the first cohort of patients receiving BM grafts MMF will be added to the Siro. For patients who develop GVHD, immunosuppressives may be continued for longer time periods according to standard of care guidelines. If no GVHD develops, immunosuppression should begin to be tapered at approximately d100 and a plan to be off immunosuppression by 9 months. Patients with persistent GVHD while on Siro, MMF and topical steroid therapies should be treated with methylprednisolone 1-2.5 mg/kg daily. This protocol offers a strategy for post transplant cyclophosphamide as GVHD prophylaxis specifically for patients at high risk of complications from the use of calcineurin inhibitors. Conditioning will be a RIC regimen.

4.2 Intervention

Patients ineligible/inappropriate for a TCD graft or at increased risk of transplant related complications associated with the use of standard calcineurin inhibitor GVHD prophylaxis who carry a diagnosis of ALL, AML, high risk forms of MDS, CML resistant to, intolerant to or progressing on TKIs, accelerated phase CML or treated blast crisis of CML, advanced MPD and NHL and who fulfill eligibility requirements and consent to treatment, will receive a RIC conditioning regimen with a 8/8 matched related or unrelated donor stem cell graft. The RIC conditioning will consist of melphalan 70 mg/m ^{2} x 2 days plus fludarabine 25 mg/m ^{2} x 5 days. GVHD prophylaxis with posttransplant cyclophosphamide 50 mg/kg/day on days +3 and +4 will be given to all patients. The de-escalation of GHVD prophylaxes for the BM and PBSC cohorts are described in the two tables below.

BM De-escalation

Level	Prophylaxis
-1	MMF + Siro+ Cyclophosphamide
1	Siro+ Cyclophosphamide
2	Cyclophosphamide

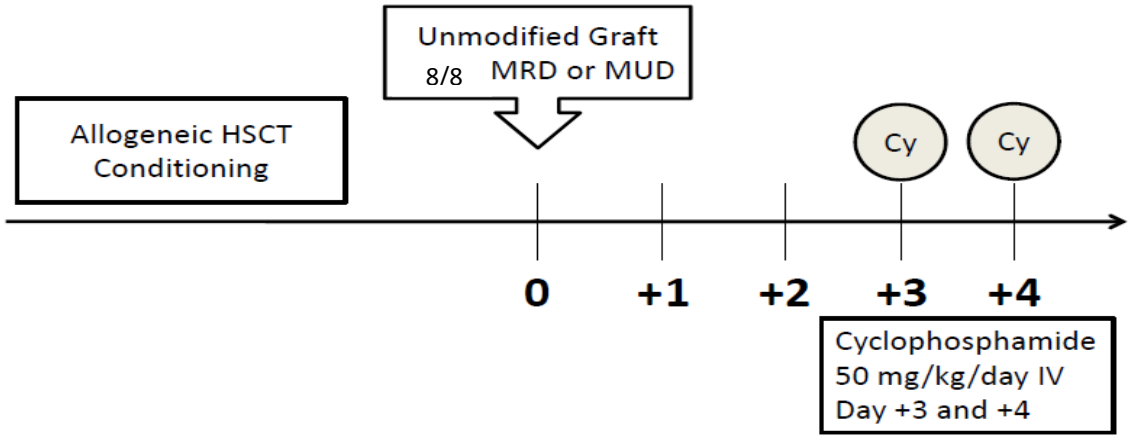
PBSC De-escalation

Level	Prophylaxis
-1	Methylprednisolone+ MMF+ Siro+ Cyclophosphamide

1	MMF+ Siro+ Cyclophosphamide
2	Siro+ Cyclophosphamide
3	Cyclophosphamide

The cell dose for BM grafts will be targeted for 4 x 10 ^{8}TNC/kg, and PBPC grafts will be capped at 3 x 10^{6} CD34/kg in order to limit the T cell dose. For the groups of patients who are to receive Siro +/- MMF, immunosuppressive drugs will begin on d+5. Siro trough level will be targeted for 3-15 mcg/ml. Mesna will be given as per MSKCC guidelines to reduce the toxicity of the cyclophosphamide. Neupogen can be given beginning at d+7.

Schema: Post transplant Cyclophosphamide GVHD Prophylaxis



Melphalan 70 mg/m ^{2}/d will be administered intravenously on d-6 and -5

Fludarabine 25 mg/m ^{2}/d will be administered intravenously on d-6 thru -2

Day -1 will be a day or rest

Cyclophosphamide and mesna will be given on d+3 and +4

Siro +/- MMF will be started in those patients who are to receive it on d+5. .

Neupogen will begin d+7.

5.0 THERAPEUTIC/DIAGNOSTIC AGENTS

5.1 *Melphalan (Alkeran®)*

Source and pharmacology: Supplier: Glaxo Wellcome. A derivative of nitrogen mustard, an analog of mustard gas. It is a polyfunctional alkylating agent that causes miscoding, cross-linkage of DNA, and single-strand breakage of DNA. It inhibits cellular glycolysis, respiration, and protein synthesis. It is cell cycle-phase non-specific.

Formulation and stability: A lyophilized powder of 50 mg melphalan and 20 mg povidone per vial. Also provided is 10 ml of sterile diluent for use in reconstituting the product and a 0.45 micron filter. The special diluent has the following composition: Sodium citrate 0.2 g, Propylene glycol 6.0 ml, Ethanol (95%) 0.5 ml, and sterile water 10 ml.

Solution preparation:

1. Vial/50 mg: Reconstitute by rapidly injecting 10 ml of the supplied diluent into the vial to yield a final concentration of 5 mg/ml.
2. Shake vigorously until the solution is clear.
3. Immediately dilute the dose to be administered in 0.9% Sodium Chloride, USP, to a concentration no greater than 0.45 mg/ml

Storage and stability: The intact packages should be stored at room temperature (15-30°C) protected from light. Shelf-life surveillance of the intact dosage form is ongoing. Constitution with the special diluent as directed results in a solution that retains at least 90% potency for about three hours at 30°C. Storage at 5°C results in precipitation.

Administration: Intravenous, over 30 minutes. Complete infusion within 60 minutes of preparation.

5.2 *Fludarabine (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.

Solution 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.
3. Supportive medications: none.

Toxicities: see Section 11.5.1

Incompatibilities: acyclovir, amphotericin B, chlorpromazine, daunorubicin, ganciclovir, hydroxyzine, miconazole, prochlorperazine.

5.3 Cyclophosphamide (Cytoxan ®)

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

Solution Preparation:

1. Standard iv fluid: D5W.
2. Final concentration range up to: 20mg/ml.

3. IV piggyback volume: for doses < 1200mg/m2, 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/m2.
2. Emetic potential: high and delayed.
3. Supportive medications: anti-emetics and mesna.

Toxicities: see Section 11.5.2
Incompatibilities: do not administer with other drugs.

5.4 Mesna (Mesnex®)

Supplied as: 200 mg/2 ml ampule, 1 gram/10 ml multi-dose vials
Reconstitution: not applicable.
Storage and stability:
1. Store vials at room temperature.
2. Multi-dose vials may be stored and used for up to 8 days after initial entry.
3. Infusions prepared in D5W are stable for 48 hours when stored under refrigeration or at room temperature.

Solution Preparation:
1. Must be diluted prior to infusion
2. Standard IV fluid: D5W.
3. IV piggyback volume: 50ml D5W

Usual Dosage and administration: 100% of the total cyclophosphamide dose divided into 3 doses, and 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: mesna functions solely for uroprotection to prevent hemorrhagic cystitis and has no cytotoxic activity.
Toxicities: none.
Incompatibilities: Carboplatin, Cisplatin, and Epirubicin.

5.5 Sirolimus

Indications: Immunosuppressant used in the prevention of graft-versus-host-disease (GVHD) following allogeneic bone marrow transplantation.
Storage and Stability: Oral solution: Store under refrigeration, 2°C to 8°C (36°F to 46°F). Protect from light. A slight haze may develop in refrigerated solutions, but the quality of the product is not affected. After opening, solution should be used in 1 month. If necessary, may be stored at temperatures up to 25°C (77°F) for ≤15 days after opening. Product may be stored in amber syringe for a maximum of 24 hours (at room temperature or refrigerated). Solution should be used immediately following dilution. Tablet: Store at room temperature of 20°C to 25°C (68°F to 77°F). Protect from light
Toxicities: see section 11.0

5.6 Mycophenolate Mofetil (CellCept®)(MMF)

Supplied as: 500 mg vial of powder for reconstitution.
Reconstitution: reconstitute each 500 mg vial with 14 ml of D5W only. Gently shake the vial to dissolve the drug. The vial will contain 500 mg of mycophenolate in approximately 15 ml.

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.

Solution 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. MMF vials are stable for 4 hours at room temperature after reconstitution.
5. Doses of MMF may begin infusion into the patient up to 4 hours after initial reconstitution of the vials.

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.

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

Supplied as: 300 mcg/ml; 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.

Storage and Stability: Store in a refrigerator (2-8°C). Do not freeze. If inadvertently the filgrastim is exposed to freezing temperatures for up to 24 hours, it may be thawed and refrigerated for use. Avoid shaking. Filgrastim may be allowed to reach room temperature for 24 hours prior to use.

Solution 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 of filgrastim in solution is between 5-15 mcg/ml, albumin 2 mg/ml must be added to the solution 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.

6.0 CRITERIA FOR SUBJECT ELIGIBILITY

6.1 Subject Inclusion Criteria

- Age: Patients over age 18 who are deemed eligible for transplant by their treating physician.
- Disease status:
  1. AML in ≥ 1st remission - excluding those in 1st remission with 'good risk' cytogenetic features (i.e. t(8;21), t(15;17), inv 16).
  2. Secondary AML
  3. ALL/LL in 1st remission with clinical or molecular features indicating a high risk for relapse; or ALL > 2nd remission

4. CML failing to respond to, progressing on or not tolerating appropriate TKI therapy in first chronic phase of disease; CML in accelerated phase, second chronic phase, or in CR after accelerated phase or blast crisis.
5. Non-Hodgkins lymphoma with chemoresponsive disease in any of the following categories:
    a. high grade lymphomas who have failed to achieve a first CR or have relapsed following a 1st remission who are not candidates for autologous transplants or transplants requiring the use of calcineurin inhibitors.
    b. any NHL with therapy responsive disease which is considered not curable outside the transplant setting and not eligible/appropriate for autologous transplant or a higher priority protocol.
6. Myelodysplastic syndrome (MDS): RA/RCMD with high risk cytogenetic features or transfusion dependence, RAEB-1 and RAEB-2 and AML evolved from MDS, who are not eligible for a higher priority protocol.
7. Chronic myelomonocytic leukemia: CMML-1 and CMML-2, advanced polycythemia vera, and myelofibrosis.
    a. Patients must have a healthy HLA compatible (8/8 molecularly matched related, or unrelated) donor willing to undergo BM harvesting or PBSC apheresis after G-CSF administration. BM will be the preferred graft source.
    b. Patients diagnosed with any form of acute leukemia must have received induction and at least one course of consolidation chemotherapy pretransplant

- Patients must have a Karnofsky Performance Status $\geq$ 70%
- Patients will have a eGFR <60 ml/min/1.73 m^2
    1. Patients must have adequate organ function measured by: Cardiac: asymptomatic or if symptomatic then LVEF at rest must be > 50% and must improve with exercise.
    2. Hepatic: ALT < 3 x ULN and total serum bilirubin < 1.5 x ULN, unless there is congenital benign hyperbilirubinemia
    3. Renal: eGFR > 30 ml/min/1.73 m^2
    4. Pulmonary*:* asymptomatic or if ^{sy}mptomatic*,* DLCO > 50% of predicted (corrected for hemoglobin)
- Each patient must be willing to participate as a research subject and must sign an informed consent form.
- Patient must have a fully matched related or unrelated donor willing to donate stem cells.

6.2 Subject Exclusion Criteria

- Major surgery or irradiation within two weeks.
- Active CNS or extramedullary malignant disease.
- Active and uncontrolled infection at time of transplantation including active infection with Aspergillus or other mold, or HIV infection
- Pregnant or lactating women – they are excluded, given the potential teratogenic effects of chemotherapy and agents used in the transplant.
- Male and female patients of child-bearing potential unwilling to use effective means of contraception
- HIV or HTLV I/II positive, hepatitis C or chronic active hepatitis B.
- Patients who have had a previous malignancy unless they are deemed by their treating physicians to be at low risk for recurrence.

- 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.

7.0 RECRUITMENT PLAN

An attending physician of the Adult BMT service will recruit and consent patients who fulfill the eligibility criteria as listed in Section 6.0 for this study. The estimated time to enroll the necessary number of patients is 5 years.

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. Pregnant women are excluded from participation in this study.

Any healthy family member will be considered for stem cell donation. Selection of a donor will be based on matching of HLA-A, B, C, and DR loci . A prospective donor must be a 8/8 HLA match (HLA-A, B, C, DR, DQ) – all typing will be determined at the DNA level. The related donors will provide signed informed consent to undergo a BM harvest or to receive a 5-6-day course of G-CSF and to undergo two PBSC leukaphereses. In addition, if the PBSC collection is determined to be inadequate, the donor should agree to undergo a BM harvest under general anesthesia if additional stem cells are needed. The related donor will undergo a medical evaluation as per the BMT Service.

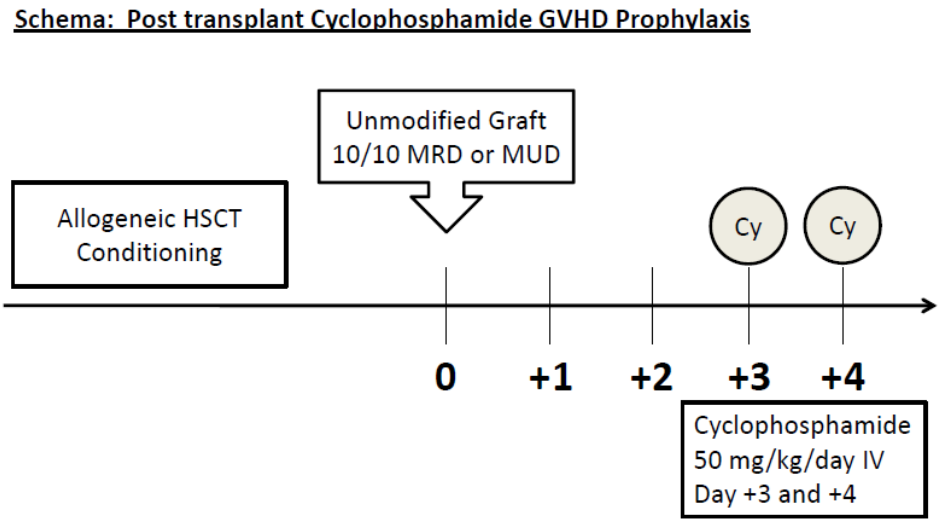
If an adequate related donor is not identified, a search will be conducted through the National Marrow Donor Program and individual registries. Screening and harvests will be conducted as per the NMDP guidelines. The unrelated donor must be a 8/8 HLA molecular match.

8.0 PRETREATMENT EVALUATION

The following tests are required within 30 days prior to beginning the conditioning regimen or sooner as clinically indicated.

- Complete history and physical examination (including Karnofsky performance status)
- CBC, comprehensive panel (defined previously), LDH, PT/PTT
- Bone marrow aspiration, with cytogenetics and molecular studies when clinically indicated. Radiologic imaging studies as clinically appropriate for diseases staging.
- Serum or urine HCG in women of childbearing potential
- EKG
- Urinalysis, eGFR and Renal Service consultation based on discussion with a Renal Service attending.
- Chest x-ray
- Red blood type and screen
- STR on donor and patient
- Serologic/PCR testing for transmittable diseases
- Stress echocardiogram or stress MUGA and PFTs

9.0 TREATMENT/INTERVENTION PLAN



9.1 RIC allogeneic HSCT conditioning

- Melphalan 70 mg/m^2 x 2 days d -6 and -5 and fludarabine 25 mg/m^2 x 5 days days -6 thru -2. There will be no adjustment of the chemotherapy based on renal function: Cell dose for BM grafts will be targeted for 4 x 10^8 TNC/kg, and PBSPC grafts will be capped at 3 x 10^6 CD34/kg
- There will be no adjustment of the chemotherapy based on renal function:
- Cell dose for BM grafts will be targeted for 4 x 10e8 TNC/kg, and PBSPC grafts will be capped at 3 x 10e6 CD34/kg.

9.2 Cyclophosphamide will be administered at 50 mg/kg/day IV on days +3 and +4 post transplantation

- Dosing will be based on ideal body weight
- Mesna will be administered IV at 80% of the cyclophosphamide dose, in 4 divided doses, on days +3 and +4 post transplantation.

9.3 Additional GVHD prophylaxis

- As shown in the tables in section 4.2, additional GVHD prophylaxes are initially provided. Siro standard starting dose consists of 6 mg PO loading dose, with a maintenance dose started 24 hours later at 2 mg PO/day. MMF dosing is 1g IV q8h for patients >50 kg. Both will begin on d+5 and will be titrated to standard trough levels.
- If no acute GVHD develops, Siro +/- MMF will begin being titrated down at d+100.

9.4 Prophylaxis against infections

- Standard of care guidelines will be followed for prophylaxis against post transplant infections by opportunistic organisms, including Pneumocystis jiroveci, fungal organisms, DNA herpesviruses and more specifically CMV.

9.5 Enrollment

- All patients may be entered into a new cohort level only after 3 patients have been treated at the previous level and survived to D+100 +/- 5 days without developing grade IV GVHD.

10.0 EVALUATION DURING TREATMENT/INTERVENTION

10.1 Post-transplant evaluation

a. Clinical Assessments: Patients will receive physical examinations daily while an inpatient with particular attention to assessment of potential toxicities induced by preparatory cytoreductive therapy, including mucositis, cystitis, sepsis, pneumonia, veno-occlusive disease (VOD) and transplant- associated complications including graft failure, acute and chronic GVHD, renal insufficiency/failure, and transplant associated infections. Patients are closely monitored for alterations in vital signs, weight, oral and intravenous intake, and intestinal and urinary output. Cardiac assessments are obtained prior to admission to assess cardiac function and thereafter when clinically indicated. Pulmonary status is closely monitored when clinically indicated by radiographic and functional analysis. Following discharge, patients will receive physical examinations during outpatient visits as described in the table below. Women at risk of menstruating will receive hormone supplementation to prevent bleeding during periods of thrombocytopenia.

Procedures	Pre-treat	Days Post-Transplant																	
		7	14	21	28	35	42	49	56	63	70	77	84	100	6 mts	9 mts	12 mts	18 mts	24 mts
Window	30	(+/-) 3								(+/-) 7					(+/-) 14		(+/-) 30		
Eligibility	X																		
Informed consent	X																		
History/Physical	X																		
Dental Evaluation																			
CBC	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Blood Chemistry/CMP (1)	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Coagulation	X																		
Blood Type and screen																			
Serology Testing (2)	X																		
Pregnancy test if applicable (childbearing age)	X																		
Urinalysis	X																		
EKG and echo or MUGA	X																		
CT chest/abd/pelvis	X																		
PFT	X																		
Chest x-ray	X																		
Bone marrow aspirate and biopsy	X				X									X	X	X*	X	X	X
Flow 7 lymphocyte panel					X									X	X	X*	X	X*	X*
Peripheral blood lymphocyte (PBL) (3)														X*	X*	X*	X	X*	X*
Chimerism (Blood and BM) (4)	X				X									X	X		X		X
Disease Assessment (5)	X				X									X	X	X	X		
GvHD evaluation			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

Toxicity assessment		X	X	X	X	X	X	X	X	X	X	X	X	X					

if clinically indicated

(1) BUN, creatinine, electrolytes, glucose, total protein, albumin, AST, ALT, bilirubin, alkaline phosphatase
(2) CMV, Herpes Zoster, Herpes Simplex, Hepatitis B, Hepatitis C, EBV, Syphilis and Toxoplasmosis
(3) In vitro response of PBL to mitogen = PHA.
(4) Chimerism at baseline (pretreatment) can be either blood or bone marrow
(5) Includes bone marrow aspirate and/or biopsy for patients with MDS, acute or chronic leukemia, CLL. Patients with lymphoma or extramedullary leukemia will undergo CT scans. Radiology disease assessments are not needed on Day 28.

11.0 TOXICITIES/SIDE EFFECTS

Toxicities will be graded per the BMT Service SAE Guide using the NCI-Common Terminology for Adverse Events (CTCAE), version 4.0. Patients recruited to this transplantation trial are individuals who are either referred by physicians or self-referred for marrow transplantation as a potentially curative treatment for their malignancy. Prior to consideration for transplant, all patients undergo a series of consultations discussing the risks and potential benefits of an allogeneic stem cell transplantation and the different procedures which will be a normal part of the transplant course. The risks and potential

benefits of the transplant procedure are also discussed. This protocol offers a strategy for post transplant cyclophosphamide as GVHD prophylaxis.

11.1 General Description of Risks to Recipients

Infections and hemorrhage constitute major and continuing risks throughout the period of marrow aplasia. These are, however, also the major risks associated with the primary disease. Certain opportunistic infections remain a risk in transplant patients beyond recovery of circulating leukocytes, for at least 9-12 months post-transplant, e.g. Pneumocystis carinii, cytomegalovirus and Epstein Barr virus.

11.2 Melphalan

- Likely side effects include myelosuppression, fatigue, not sleeping well, anorexia, nausea, vomiting, diarrhea, mucositis weight gain and swelling, changes in blood sodium level, alopecia, needing transfusions of platelets and red blood cells, fever, needing antibiotics to treat infection, transient liver dysfunction.
- Less likely side effects include Late effects of these chemotherapy agents include: cataracts and under-activity of the thyroid gland. Both of these side effects can be easily treated. Renal or bladder dysfunction (increased BUN, creatinine, necrosis) may be seen.
- Rare but serious side effects include pulmonary fibrosis, respiratory distress has been rarely reported. Serious hypersensitivity reactions: Edema, rash, anaphylaxis

11.3 Fludarabine

- Likely side effects include Myelosuppression, tiredness, not sleeping well, anorexia, nausea, vomiting, diarrhea, mucositis weight gain and swelling, changes in blood sodium level, alopecia, needing transfusions of platelets and red blood cells, fever, needing antibiotics to treat infection, jaundice and elevations of liver enzymes
- Less likely side effects include late effects of these chemotherapy agents include: cataracts and under-activity of the thyroid gland. Both of these side effects can be easily treated. confusion, numbness, loss of vision, loss of balance, difficulty walking

11.4 Sirolimus

- Likely side effects include immune suppression which leads to increased risk for infection. This is managed with aggressive supportive care with both prophylaxis and treatment of infectious complications. hypertriglyceridemia, Hypercholesterolemia, mild thrombocytopenia, anemia, leukopenia, hypokalemia, elevated LDH, arthralgia, epistaxis, edema, and infections.
- Rare but serious side effects a syndrome of thrombotic microangiopathy, comprised of microangiopathic hemolytic anemia, thrombocytopenia and renal dysfunction has been described in association with Siro and tacrolimus use.

11.5 Mycophenolate

- Likely side effects include immune suppression which leads to increased risk for infection. This is managed with aggressive supportive care with both prophylaxis and treatment of infectious complications.
- Less likely side effects include myelosuppression, headache, insomnia, aches and pains, rash, nausea, anorexia and diarrhea.
- Less likely side effects include 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.

Likely

Reproductive risks: Sterility. Male patients may be offered sperm banking before admission for the transplant. Possibilities of preserving the ability to have children for female patients can be discussed with the doctor. Patients should not become pregnant or father a baby while on this study because the drugs in this study can affect an unborn baby. Women should not breast feed a baby while on this study. A pregnancy test is required of all females of childbearing age before starting the transplant.

Growth factor (G-CSF): Bone pain, headache, body ache, feeling tired, swelling of hands/feet, nausea. These are generally mild and will go away when the growth factor is stopped.

Transplant related risks

Blood transfusions: Transfusions may induce allergic reactions. Small, subclinical pulmonary emboli may occur, but these rarely if ever require any intervention. Standard pre-medications for blood products may be used before administration of the marrow graft. Fluid overload can be managed with diuretics. Allergic reactions of variable severity can be prevented or mitigated by premedication with antipyretics, antihistamines, and narcotics. These products may also serve as vectors of serious infection (e.g., CMV, hepatitis, AIDS). To circumvent this, prospective blood and marrow donors will be screened per AABB and FAHCT guidelines. CMV antibody (-) blood products will be used in CMV(-) individuals, whenever possible, regardless of the antibody status of the marrow donor. All blood products are irradiated (3000r, ^{137}Cs) to circumvent the risk of GvHD caused by contaminating lymphocytes in the transfused fractions.

Receiving BM or PBSC: The volume of the graft infused can be up to approximately 1500 cc. Possible side effects include: changes in blood pressure, fever, headache, shortness of breath, chills, sweats, nausea/vomiting, bad taste in the mouth. Pre-medications are given to reduce these side effects. Reactions will be treated as per standard MSKCC guidelines.

Graft-versus-host-disease (known as GvHD): This condition happens when the transplanted donor cells recognizes the patient's body as foreign and attacks it. Approx. 30-50% of patients receiving conventional grafts will develop grade II-IV acute GvHD. GvHD can be treated with medications (either IV or tablets). A biopsy may be necessary to make the diagnosis of GvHD.

Acute GvHD usually occurs in the first 3 months or as immune suppressive medications are tapered and may cause: skin rashes, nausea, vomiting, diarrhea, hepatitis, increased risk of infection, ulceration of the surfaces of the oral cavity, esophagus, and intestines, and suppressed or delayed recovery of the hematopoietic and immune system. The incidence in previous reports using BM is approx. 50%, the incidence may be higher using PBPC grafts.

Chronic GvHD can occur any time after the first 3 months. Approximately 50% of patients with acute GvHD may also develop chronic GvHD, manifested to varying degrees by scleroderma-like changes of the skin, cirrhosis of the liver, sclerosis of lacrimal and salivary ducts, chronic inflammation and scarring of the gastrointestinal tract with consequent malabsorption and diarrhea, chronic bronchitis, and suppression of the immune system. This can be treated with standard or protocol-based experimental immunosuppression, but may be refractory.

Severe GVHD: Rarely, GvHD can be severe or deadly. Severe acute GvHD could involve a severe skin rash like a burn, severe vomiting and/or diarrhea, liver failure and infections or bleeding. Severe acute GvHD will be treated with intense immunosuppressive therapy according to standard clinical practice or other experimental protocol. Severe chronic GvHD could involve similar symptoms but may produce other symptoms such as severe skin changes, severe dry eyes and weight loss.

Steroids, as treatment for GvHD: inability to sleep, high blood sugar, puffiness of the face, changes in the skin, high blood pressure, increased risk of infection, weight gain, reduced growth in children, thinning of the bones

Infections or bleeding: Full recovery of blood counts may take months. Full recovery of the immune system may take months to a few years. For this reason patients will be at increased risk of infections and bleeding. Medications are given to reduce the chance of those infections. Patients will receive treatment if they do get an infection and most infections can be treated successfully with antibiotics. Patients will stay in the hospital longer or be readmitted if found to have an infection. Patients are watched closely for bleeding and given platelet transfusions to prevent serious bleeding, but minor bleeding may occur.

Serious infections or bleeding: Some infections are very difficult to treat, even with strong antibiotics. Rarely, serious infections can be passed on by the transfusion of blood products. Serious bleeding can happen in spite of platelet transfusions. Rarely infections or bleeding are lethal.

Pneumocystis jiroveci prophylaxis: The risk of trimethoprim and sulfamethoxazole in the doses given are primarily hypersensitivity reactions and signs of folate deficiency. Any patient with known hypersensitivity to these compounds will not receive these drugs. The risks of parenteral pentamidine are primarily hypotension and hypoglycemia both of which will be monitored during and following administration of the drug. Hypokalemia or hypomagnesemia associated with prolonged QT syndrome or Torsade de pointe necessitates strict electrolyte monitoring. The risks of aerosolized pentamidine are mild bronchospasm primarily observed in (prior) tobacco abusers and easily managed with bronchodilator therapy.

Risk of a secondary cancer may occur after chemotherapy. The risk of developing a secondary cancer of the skin, cervix, etc., which has been seen in other transplant studies is less than 5%.

Graft Failure/rejection: Stem cell grafts may fail to grow or may start to grow and then be rejected by the patient's immune system. Past experience with this type of transplant using

BM suggests that this may occur in ~10% of patients. The incidence using PBSC has not been reported. If graft failure occurs, it is unlikely that bone marrow will recover and a second transplant with stem cells from the same donor or a different donor will be needed.

Progression of Disease or Relapse is a risk even if the transplant is initially successful.

Worsening Kidney Function: Since the patients on this protocol will have some degree of renal insufficiency and despite avoidance of nephrotoxic medications, there is a risk that the kidney function will deteriorate and the patient may require dialysis.

Death: There is an approximately 5-10% risk of treatment related mortality within the first month of transplant for patients with normal renal function due to the risk of severe regimen related toxicity, hemorrhage, opportunistic infection, or other complications. Patients enrolled on this protocol may be at higher risk of complications due to their baseline renal insufficiency.

12.0 CRITERIA FOR THERAPEUTIC RESPONSE/OUTCOME ASSESSMENT

Definition of events in the post-transplant course important for analysis and treatment.

12.1 Engraftment and Chimerism

Engraftment will be documented by analysis of blood cells, T cells and bone marrow cells for chimerism by standard cytogenetic studies at about 1 month, 100 days, 6 months, 9 months (if clinically indicated), and 12 months, 18 months (if clinically indicated), and 24 months post transplant or as needed thereafter.

12.2 Graft Failure or rejection

Primary non-engraftment is diagnosed when the patient fails to achieve an ANC 500/mm3 at any time in the first 28 days post-transplant. If the patient's leukemia recurs during this interval, the patient is scored as having refractory leukemia. In such a situation, the absence of donor hematopoiesis is not evaluable for graft failure or rejection. If host T-cells capable of specifically inhibiting donor hematopoietic progenitor growth in vitro are concurrently detected during graft failure, a presumptive diagnosis of immune mediated rejection is made: if (1) after achievement of an ANC 500/mm_3, the ANC declines to <500/mm_3 for more than 3 consecutive days in the absence of relapse, or, (2) there is absence of donor cells in the marrow and/or blood as demonstrated by chimerism assay in the absence of relapse, a diagnosis of secondary graft failure is made. If, however, recurrence of host leukemia is detected concurrently, the patient is not evaluable for graft failure or rejection.

Patients with evidence of graft failure without evidence of recurrence of host leukemia will have additional studies drawn to ascertain cause and define relevant histoincompatibilities. These analyses may include (1) Evaluation of bone marrow aspirates and biopsies for residual or recurrent leukemia and chimerism, when indicated, (2) Culture and/or molecular analyses of marrow and blood for viral pathogens potentially causing graft failure including CMV, HHV6 and parvovirus B 19, (3) Immunophenotypic and genetic analysis of circulating T-cells and NK cells to ascertain their origin and potential function, (4) Analysis of the functional activity of residual circulating lymphocytes Patients who suffer graft failure will be considered for a secondary transplant. The need for additional immunosuppression or

treatment for viral infection prior to the secondary transplant will be determined by the results obtained from chimeric and viral studies.

12.3 Graft-versus-host-disease

Standard BMT-CTN and IBMTR systems clinical criteria as defined by Rowlings, et al (55) will be used to establish and grade acute GVHD.

To determine the severity of acute GVHD, data will be collected approximately weekly at least during the first 100 days (as per Adult BMT Service guidelines) to characterize the severity of symptoms and signs caused by GVHD and to evaluate possible confounding factors.

Patients will be observed for acute and/or chronic GVHD as long as they have not received donor derived leukocytes infusions (DLI) for the treatment of relapse or infections. If at any time, a patient receives DLI, that time will represent the end-time for evaluation of GVHD. Graft-versus-host disease occurring after DLI infusions will be analyzed separately.

Patients with moderate to severe acute GVHD (grade II-IV) will be treated as per Adult BMT Service guidelines Patients failing to respond to systemic steroids will be considered for treatment with experimental treatments available at the time of diagnosis of GVHD.

Chronic GVHD will be diagnosed and graded according to the (NIH criteria) treated with standard or experimental immunosuppressive therapy.

12.4 Infections

The occurrence of life-threatening opportunistic infections will be evaluated according to the criteria established by BMT CTN (see Appendix 2), and will be correlated with the level of immune recovery. The infection-related mortality will also be monitored. Patients will be considered to have died from infection if death is attributed to a recent severe infection and/or infection was identified at autopsy. Patients with relapsed disease before death will be excluded from the above definition, even if an infection was the final cause of death.

12.5 Disease Relapse

Relapse of MDS, AML and AML will be defined by an increasing number of blasts in the marrow over 5%, by the presence of circulating peripheral blasts, by the presence of blasts in any extramedullary site, or recurrence of a cytogenetic abnormality. Relapse of NHL will be determined by bone marrow evaluation (when appropriate) and imaging studies similar to those used for pretransplant disease evaluation.

12.6 Disease-free survival

DFS is defined as the minimum interval of time to relapse/recurrence, to death or to the last follow-up, from the time of transplant.

12.7 Overall survival

Overall survival is defined as time from transplant to death or last follow-up.

12.8 Renal insufficiency

Renal insufficiency is defined as a calculated eGFR <60 ml/min/1.73m^2. Those with a eGFR < 30 ml/min/1.73m^2 will be considered ineligible.

13.0 CRITERIA FOR REMOVAL FROM STUDY

If at any time the research participant is found to be ineligible for the protocol as designated in the section on Criteria for research participant eligibility (eg a change in diagnosis), the research participant will be removed from the study. Also research participants may be removed from the study if requested by the research participant. Management will depend on where they are in their treatment course. Such research participants will receive appropriate supportive care.

14.0 BIOSTATISTICS

This is a pilot study to investigate the safety of using post transplant Cyclophosphamide as acute GVHD prophylaxis in patients who cannot receive calcineurin inhibitors. The outcome for this study is day +100 grade III-IV acute GVHD. The study will be separated into two cohorts determined by whether a BM or PBSC graft will be used. Both cohorts will receive additional prophylaxes initially which can be removed if excessive grade III-IV GVHD at day +100 is not observed. When applicable, the de-escalation schema will follow a 3-by-3 strategy typically used in dose escalation studies. Since this study includes a patient population previously ineligible for transplant, it is challenging to provide an accrual rate. However, there may be approximately 25 patients or more annually eligible for inclusion on this study, of whom 5-10 may receive a BM graft and 15-20 may receive a PBSC graft.

BM Graft:

The initial cohort of 3 patients who receive a bone marrow graft will receive both Siro and cyclophosphamide. If no patient in the initial three develop grade III-IV GVHD, an additional three patients will be treated with the combination for safety confirmation.

The trial for this cohort will enroll between 2 to 6 patients. De- escalation of prophylaxes may occur at a later time point if excessive GVHD is not observed at this level.

There will be six patients treated at the final combination of prophylaxses. In this cohort, the grade III-IV GHVD estimate and corresponding 95% exact confidence interval will be 0% (0%-46%) if no patient has GVHD and will be 17% (0.4%-64%) if one patient have GHVD.

PBSC Graft:

The initial cohort of 3 patients will receive Siro plus MMF and cyclophosphamide (level 1 in table below). The dose de-escalation scheme is as follows:

1. If no patient in the initial 3 experiences grade III-IV GVHD at a given level, the next level will be studied.
1. If one of the initial 3 experiences grade III-IV GVHD, 3 additional patients will be treated at that level.

2. If 2 or more patients experience DLTs at a given level, the previous level will be declared the prophylaxis strategy.
3. In order to decide on a final prophylaxis strategy, a total of six patients must be treated at that level.

The trial for this cohort will enroll between 2 to 12 patients. De- escalation of prophylaxes will only occur once all patients at the current level have cleared the day +100 window without grade III-IV GVHD

PBSC De-escalation

Level	Prophylaxis
1	MMF+ Siro+ Cyclophosphamide
2	Siro+ Cyclophosphamide

Using this de-escalation scheme, de-escalation to the next level is probable if the risk of grade III-IV GVHD is low, and the likelihood of de-escalation decreases as the risk of grade III-IV GVHD increases, as demonstrated in table below:

De-Escelation / GVHD Risk						
True risk of grade III-IV GVHD	.10	.20	.30	.40	.50	.60
Probability of De-Escelation	.91	.71	.49	.31	.17	.08

Treatment-related mortality will be closely monitored in this high-risk patient cohort. In order to reduce patient risk, the following trial suspension rules will be implemented. Should more than 2 out of the six patients in the BM cohort die of TRM by day +100, accrual in that cohort will stop. Should more than 2 out of the first six patients or four at any point in the PBSC cohort die of TRM by day +100, accrual in that cohort will stop.

In addition to TRM, this trial may be temporarily suspended to evaluate the overall treatment strategy if >2 patients require post-transplant dialysis at any point post transplant in the combined BM and PBSC cohorts.

There are a number of secondary objectives included in this protocol. These descriptive objectives will be evaluated separately for patients receiving BM and PBPC grafts.

1. Cumulative incidence functions (CIF) will be used to estimate 2-year acute and chronic GVHD.
2. 100-day TRM will be estimated using CIF.
3. The incidence of graft failure will be evaluated using CIF.
4. Overall and disease-free survival will be evaluated using Kaplan-Meier methodology.
5. The proportion of patients with acute or worsening chronic renal insufficiency will be tabulated. Worsening chronic insufficiency is evaluated as the change in creatinine from the time of transplant.
6. The proportion of patients who have infectious complications will be tabulated.
7. Time to immune reconstitution will be summarized using CIF.

15.0 RESEARCH PARTICIPANT REGISTRATION AND RANDOMIZATION PROCEDURES

15.1 Research Participant Registration

Confirm eligibility as defined in the section entitled Criteria for Patient/Subject Eligibility.

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.

All participants must be registered through the Protocol Participant Registration (PPR) Office at Memorial Sloan-Kettering Cancer Center. PPR is available Monday through Friday from 8:30am – 5:30pm at 646-735-8000. Registrations must be submitted via the PPR Electronic Registration System (http://ppr/). The completed signature page of the written consent/RA or verbal script/RA, a completed Eligibility Checklist and other relevant documents must be uploaded via the PPR Electronic Registration System.

15.2 Randomization

This research study does not require a randomization.

16.0 DATA MANAGEMENT ISSUES

A Research Study Assistant (RSA) will be assigned to the study and will be responsible for patient accruals. The responsibilities of the RSA and principal investigator include project compliance, data collection, abstraction and entry, data reporting, regulatory monitoring, problem resolution and prioritization, and coordinate the activities of the protocol study team. The data collected for this study will be entered into The Clinical Research Data Base (CRDB), a secure database. Source documentation will be available to support the computerized patient record.

16.1 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.

16.2 Data and Safety Monitoring

The Data and Safety Monitoring (DSM) Plans 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 Office of Clinical Research. 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.

17.0 PROTECTION OF HUMAN SUBJECTS

The risks associated with the transplant are those associated with the toxicities of the conditioning regimens, as well as the risks of an allogeneic transplant, particularly graft failure, or graft vs. host disease, as detailed in Section 11.0.
To protect against the toxicities of the cytoreductive regimens, the patient will be transplanted in a single room, HEPA filtered environment. Organ toxicities such as mucositis, enteritis and hepatic dysfunction as well as infectious complications will be treated by standard procedures developed for transplantation to support the patient. Blood and platelet counts will be supported by transfusion. Graft failure might necessitate a second transplant, after additional conditioning. Approaches to the diagnosis and treatment of graft failure that secure consistent engraftment have been developed by the transplantation services. Similarly, advanced treatments will be instituted in the event the patient develops graft vs. host disease.

Despite a transplant, the patient’s disease may recur. In this case, standard and/or experimental therapies, such as phase I/II drugs, antibodies or cell therapies, will be available to the patient for consideration as treatment options.

Benefits:
A transplant is administered with curative intent. The approaches being evaluated may achieve this goal and may also be effective in preventing acute and chronic graft vs. host disease and providing transplantation as a potentially curative therapy for patients with renal insufficiency.

The results of this study will also define risks and benefits of high dose cyclophosphamide as GVHD prophylaxis for patients not appropriate for TCD transplantation or conventional grafts using standard calcineurin inhibitor prophylaxis. This may greatly accelerate further development of transplantation approaches employing this approach to GVHD prophylaxis.

Consent Process: Participation in this study is voluntary. All patients will be required to sign a statement of informed consent which must conform to MSKCC IRB guidelines.

Alternatives: Enrollment in this study is voluntary. Alternative treatment options will be presented to the patient prior to taking part in this study. Alternative treatment options may

include receiving treatment for the cancer with either chemotherapy or a transplant without being on a study; taking part in another study; or taking no treatment.

Costs: The patient's health plan/insurance company will need to pay for all of the costs of treatment in this study. The patient will be responsible for the costs of standard medical care, all hospitalizations and any transplant complications. Pre-authorization for the transplant will be cleared with the health plan/insurance company prior to admission. Patients will not be paid for taking part in this study.

Confidentiality: Every effort will be made to maintain patient confidentiality. Research and hospital records are confidential.

17.1 Privacy

It is the responsibility of the Research Staff to ensure that protocol subjects receive the Center's Notice of Privacy Practices. If the subject has not received one, MSK personnel must provide a Notice of Privacy Practices and obtain acknowledgment before the subject participates in the study. 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).

17.2 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

17.2.1

This research study does not require an IND approval.

18.0 INFORMED CONSENT PROCEDURES

The individual listed as consenting professionals have completed the mandatory Human Subjects Education and Certification Program. The consulting BMT attending will obtain consent from the patient or the patient's guardian. The patient will sign three copies of the

consent. One copy will be given to the patient to keep, one will be scanned into the patient's medical record and one copy will be stored in the patient's research file.

18.1 Research Authorization

Procedures for obtaining Research Authorization: Before any protocol-specific procedures are carried out, investigators and/or designated staff will fully explain the details of the protocol, study procedures, and the aspects of patient privacy concerning research specific information. In addition to signing the IRB Informed Consent, all patients must sign the Research Authorization component of the informed consent form. The Research Authorization requires a separate set of signatures from the patient. The original signed documents will become part of the patient's medical record, and each patient will receive a copy of the signed documents.

19.0 REFERENCES

1. Storb R, Antin JH, Cutler C. Should methotrexate plus calcineurin inhibitors be considered standard of care for prophylaxis of acute graft-versus-host disease? Biol Blood Marrow Transplant. 2010;16:S18-27.
2. Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. Blood. 1998;92:2303-2314.
3. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. Blood. 2000;96:2062-2068.
4. Ho VT, Weller E, Lee SJ, Alyea EP, Antin JH, Soiffer RJ. Prognostic factors for early severe pulmonary complications after hematopoietic stem cell transplantation. Biol Blood Marrow Transplant. 2001;7:223-229.
5. Antin JH, Ferrara JL. Cytokine dysregulation and acute graft-versus-host disease. Blood. 1992;80:2964-2968.
6. Chao NJ, Chen BJ. Prophylaxis and treatment of acute graft-versus-host disease. Semin Hematol. 2006;43:32-41.
7. Pidala J, Anasetti C, Jim H. Quality of life after allogeneic hematopoietic cell transplantation. Blood. 2009;114:7-19.
8. D'Cruz LM, Klein L. Development and function of agonist-induced CD25+Foxp3+ regulatory T cells in the absence of interleukin 2 signaling. Nat Immunol. 2005;6:1152-1159.
9. Fontenot JD, Rasmussen JP, Gavin MA, Rudensky AY. A function for interleukin 2 in Foxp3-expressing regulatory T cells. Nat Immunol. 2005;6:1142-1151.
10. Baan CC, van der Mast BJ, Klepper M, et al. Differential effect of calcineurin inhibitors, anti-CD25 antibodies and rapamycin on the induction of FOXP3 in human T cells. Transplantation. 2005;80:110-117.
11. Zeiser R, Nguyen VH, Beilhack A, et al. Inhibition of CD4+CD25+ regulatory T-cell function by calcineurin-dependent interleukin-2 production. Blood. 2006;108:390-399.
12. Hess AD. Modulation of graft-versus-host disease: role of regulatory T lymphocytes. Biol Blood Marrow Transplant. 2006;12:13-21.
13. Reisner Y, Kapoor N, Kirkpatrick D, et al. Transplantation for acute leukaemia with HLA-A and B nonidentical parental marrow cells fractionated with soybean agglutinin and sheep red blood cells. Lancet. 1981;2:327-331.
14. Wagner JE, Santos GW, Noga SJ, et al. Bone marrow graft engineering by counterflow centrifugal elutriation: results of a phase I-II clinical trial. Blood. 1990;75:1370-1377.

15. Waldmann H, Polliak A, Hale G, et al. Elimination of graft-versus-host disease by in-vitro depletion of alloreactive lymphocytes with a monoclonal rat anti-human lymphocyte antibody (CAMPATH-1). Lancet. 1984;2:483-486.
16. Soiffer RJ, Fairclough D, Robertson M, et al. CD6-depleted allogeneic bone marrow transplantation for acute leukemia in first complete remission. Blood. 1997;89:3039-3047.
17. Wagner JE, Thompson JS, Carter SL, Kernan NA. Effect of graft-versus-host disease prophylaxis on 3-year disease-free survival in recipients of unrelated donor bone marrow (T-cell Depletion Trial): a multi-centre, randomised phase II-III trial. Lancet. 2005;366:733-741.
18. Kernan NA, Collins NH, Juliano L, Cartagena T, Dupont B, O'Reilly RJ. Clonable T lymphocytes in T cell-depleted bone marrow transplants correlate with development of graft-v-host disease. Blood. 1986;68:770-773.
19. Lowenberg B, Wagemaker G, van Bekkum DW, et al. Graft-versus-host disease following transplantation of 'one log' versus 'two log' T-lymphocyte-depleted bone marrow from HLA-identical donors. Bone Marrow Transplant. 1986;1:133-140.
20. Papadopoulos EB, Carabasi MH, Castro-Malaspina H, et al. T-cell-depleted allogeneic bone marrow transplantation as postremission therapy for acute myelogenous leukemia: freedom from relapse in the absence of graft-versus-host disease. Blood. 1998;91:1083-1090.
21. Handgretinger R, Klingebiel T, Lang P, et al. Megadose transplantation of purified peripheral blood CD34(+) progenitor cells from HLA-mismatched parental donors in children. Bone Marrow Transplant. 2001;27:777-783.
22. Jakubowski AA, Small TN, Young JW, et al. T cell depleted stem-cell transplantation for adults with hematologic malignancies: sustained engraftment of HLA-matched related donor grafts without the use of antithymocyte globulin. Blood. 2007;110:4552-4559.
23. Urbano-Ispizua A, Rozman C, Pimentel P, et al. Risk factors for acute graft-versus-host disease in patients undergoing transplantation with CD34+ selected blood cells from HLA-identical siblings. Blood. 2002;100:724-727.
24. Devine S SR, Pasquini M, et al. HLA-identical sibling matched, CD34+ selected, T cell depleted peripheral blood stem cells following myeloablative conditioning for first or second remission acute myeloid leukemia (AML): Results of blood and marrow transplant clinical trials network (BMT CTN) Protocol 0303. Blood;114:273.
25. Aversa F, Terenzi A, Tabilio A, et al. Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse. J Clin Oncol. 2005;23:3447-3454.
26. Luznik L, Bolanos-Meade J, Zahurak M, et al. High-dose cyclophosphamide as single-agent, short-course prophylaxis of graft-versus-host disease. Blood. 2010;115:3224-3230.
27. Luznik L, Engstrom LW, Iannone R, Fuchs EJ. Posttransplantation cyclophosphamide facilitates engraftment of major histocompatibility complex-identical allogeneic marrow in mice conditioned with low-dose total body irradiation. Biol Blood Marrow Transplant. 2002;8:131-138.
28. Luznik L, Jalla S, Engstrom LW, Iannone R, Fuchs EJ. Durable engraftment of major histocompatibility complex-incompatible cells after nonmyeloablative conditioning with fludarabine, low-dose total body irradiation, and posttransplantation cyclophosphamide. Blood. 2001;98:3456-3464.
29. Luznik L, O'Donnell PV, Symons HJ, et al. HLA-haploidentical bone marrow transplantation for hematologic malignancies using nonmyeloablative conditioning and high-dose, posttransplantation cyclophosphamide. Biol Blood Marrow Transplant. 2008;14:641-650.
30. O'Donnell PV, Luznik L, Jones RJ, et al. Nonmyeloablative bone marrow transplantation from partially HLA-mismatched related donors using posttransplantation cyclophosphamide. Biol Blood Marrow Transplant. 2002;8:377-386.
31. Schleuning M, Judith D, Jedlickova Z, et al. Calcineurin inhibitor-free GVHD prophylaxis with sirolimus, mycophenolate mofetil and ATG in Allo-SCT for leukemia patients with high relapse risk: an observational cohort study. Bone Marrow Transplant. 2009;43:717-723.

32. Ciurea SO, Mulanovich V, Saliba RM, et al. Improved early outcomes using a T cell replete graft compared with T cell depleted haploidentical hematopoietic stem cell transplantation. Biol Blood Marrow Transplant 2012; 18:1835-1844.

33. Castagna L, Crocchiolo R, Furst S, et al. Bone marrow compared with peripheral blood stem cells for haploidentical transplantation with a nonmyeloablative conditioning regimen and post-transplantation cyclophosphamide. Biol Blood Marrow Transplant 2014;20:724-729.

34. Solomon ST, Sanacore M, Zhang X, et al. Calcineurin inhibitor-free graft-versus-host disease prophylaxis with post-transplntation cyclophosphamide and brief-course sirolimus followijng reduced-intensity peripheral blood stem cell transplantation. Biol Blood Marrow Transplant 2014; July 20. [Epub ahead of print].

20.0 APPENDICES

Appendix 1. BMT CTN Severity of Infection