**UNIVERSITY OF MINNESOTA BLOOD AND MARROW TRANSPLANTATION PROGRAM**

# A STUDY OF CYCLOPHOSPHAMIDE, FLUDARABINE AND ANTI-THYMOCYTE GLOBULIN FOLLOWED BY MATCHED SIBLING DONOR HEMATOPOIETIC CELL TRANSPLANTATION IN PATIENTS WITH FANCONI ANEMIA
**CPRC# MT2000-09**

Version Date
June 3, 2019

Study Committee

Margaret L. MacMillan, MD – PI
John E. Wagner, MD - Co-Investigator
Todd Defor, MS - Biostatistician

| DATE | PROTOCOL CHANGE |
|---|---|
| August 7, 2012 | Section 10.4 and 10.5 - Set enrollment at an additional 20 patients beginning with the August 2012 revision and update study stopping rules accordingly<br>Resolve data collection inconsistencies:<br>• Clarify the secondary objective/endpoint of regimen related toxicity is based on transplant outcomes through day 100 (engraftment, infections, treatment related mortality, etc.)<br>• Patients will be monitored for reportable events according to the table in section 9.1.2<br>• Add arm 4 for registration purposes – Sibling donor, without use of the CliniMACS |
| March 3, 2011 | Added documentation that protocol is conducted under IND 14536 |
| October 8, 2010 | Remove IDE references and restore protocol to standard of care; Section 9 - update UPIRTSO reporting requirements to U of MN IRB. |
| August 25, 2010 | Revision in conjunction with submission of IDE application for CliniMACS® Cell Selection System to be used for bone marrow processing.<br>Establish study arms based on cell source as follows:<br>**arm 1**: bone marrow processed using Isolex 300i (for patients enrolled through April 2010), **arm 2:** UCB (no processing), **arm 3**: bone marrow processed using CliniMACS (for patients enrolled beginning with the August 2010 protocol version); update protocol to reflect IDE reporting requirements; Section 4 - update background with interim results (for arm 1); section 7 – update to reflect change in cell processing device; added targeted event form.<br>Other revisions: Section 6.2 - update MMF administration; Section 6.4 add cell infusion guidelines; Section 10 – Statistical Considerations rewrite to reflect current study<br>Updates: Add study synopsis/schema; Section 2.2 – delete secondary objective 2.2.2. relapse in patients with myelodysplastic syndrome (MDS) or acute myeloid leukemia (AML), as these patients are not eligible as of November 2002; Section 5.3 add patient registration information; Section 6 add standard care language (delete supportive care appendix, add appendix V - Drug Formulation and Availability); Section 8 and Appendix I – update required observations (delete panorex, add CT abd, pelvis to r/o tumors, delete day 60 bm bx); Section 9 – update to current definition of UPRITSO and AE reporting table; add data collection and record retention sections; update header and footer. |
| March 24, 2010 | Section 6.2, MMF will now be used for GVHD prophylaxis per current institutional standards; Section 7.1 Cell Processing – replace Isolex 300i System with CliniMACS® Cell Selection System (Miltenyi Biotec), add protocol to current CliniMACS IDE (D. Weisdorf sponsor); add section SAE reporting and DSMP; Appendix VI - update risks. Deleted appendix VII – study uses CTC version 2 for toxicity criteria; Additional Minor edits; Update consents |
| November 5, 2007 | Further revised information on fungal prophylaxis to allow for changes based on individual patient needs. |
| September 7, 2007 | Changed fungal prophylaxis from Itraconazole to voriconazole (Section 6.2.1)<br>Dr. Norma Ramsay removed from study committee.<br>Each patient is to receive 1.0 x 105/kg CD3+ cells. An add back infusion of CD3+ cells may be required to achieve this prescribed cell dose (section 7.1.1). |
| November 24, 2003 | Maximum enrollment raised to 24 subjects and statistical section revised accordingly (section 9). |
| September 17, 2003 | Dr. Stella Davies and Dr. Anne Goldman removed from study committee. Todd Defor added as biostatistician. |
| November 20, 2002 | Revised eligibility to include **only** patients with HLA-matched sibling donors, and exclude patients with advanced MDS or AML prior to transplantation (section 5.1 and 5.2) |
| July 1, 2002 | 1. Added Amendment page<br>2. Removed the creatinine clearance > 40 mL/min from the inclusion criteria (section 5.4.1) because this study is the safest preparative regimen for Fanconi patients with matched siblings, who have severe renal disease |
| January 9, 2002 | Changed title from *A Phase I-II Study of Cyclophosphamide, Fludarabine, and Antithymocyte Globulin Followed by Matched Sibling Donor Hematopoietic Cell Transplantation in Patients with Fanconi Anemia* to *A Study of Cyclophosphamide, Fludarabine, and Antithymocyte Globulin Followed by Matched Sibling Donor Hematopoietic Cell Transplantation in Patients* |

| DATE | PROTOCOL CHANGE |
|---|---|
| | *with Fanconi Anemia* |
| November 1, 2016 | Updated protocol to remove IND as all subjects will now be co-enrolled on the HUD protocol.<br>Related donors will now be co-enrolled on MT2012-14<br>Antifungal therapy updated to current standard of care (section 6.3)<br>Removed standard of care evaluations from the trial (section 8 and Appendix 1)<br>Removed day 21 bone marrow biopsy |
| June 3, 2019 | Revised to clarify that post-transplant bone marrow aspirates and biopsies will only be performed if clinically indicated<br>Section 5.1.3, eligibility criteria: definition of moderately severe SAA will no longer be different for patients under age 18<br>Updated required observations (Section 8.0 and appendix I) to current standard of care<br>Updated reporting guidelines (Section 9) to current SOPs<br>Throughout protocol minor edits for spelling and clarity |

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## SYNOPSIS/SCHEMA

**Study Design:**

Single institution phase II study grouped by cell source:
Arm 1: match sibling donor marrow TCD using Isolex®300i System (for patients enrolled through April 2010)
Arm 2: UCB (no processing, co-enroll on MT2011-13R for unlicensed UCB registry
Arm 3: match sibling donor marrow TCD using CliniMACS Cell Selection System (effective with August 2010 protocol). Effective with November 1, 2016 protocol co enroll on MT2015-31 for TCD.
Arm 4: match sibling donor marrow without use of the CliniMACS

Except for cell source, all aspects of the study treatment are identical for all patients.

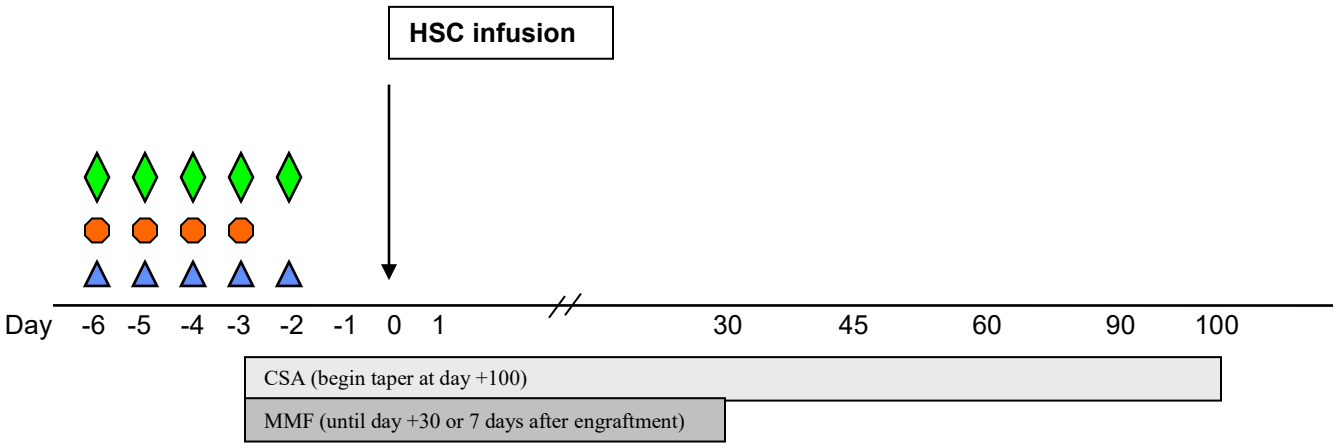
**Eligible Diseases:**

Age < 60 and diagnosis of Fanconi anemia meeting one of the following disease requirements:

- < 18 years of age with moderately severe aplastic anemia (AA) and having at least one of the following:
  - platelet count <40 x $10^9$/L
  - absolute neutrophil count <10 x $10^8$/L
  - hemoglobin <9 g/dL
- ≥18 and < 60 years of age with moderately severe aplastic anemia (AA) and having at least one of the following:
  - platelet count <20 x $10^9$/L
  - absolute neutrophil count <5 x $10^8$/L
  - hemoglobin <8 g/dL
- early myelodysplastic syndrome with multilineage dysplasia and < 5% blasts, with or without chromosomal anomalies

## SCHEMA

Patients will receive antifungal prophylaxis beginning 1 month prior to conditioning therapy, if possible.




Fludarabine 35 mg/m$^2$ IV

Cyclophosphamide 5 mg/kg IV

Anti-thymocyte globulin (ATG) 30 mg/kg with Methylprednisolone 2 mg/kg/day as pre-med

## 1.0 TITLE

A study of cyclophosphamide (CY), fludarabine (FLU) and anti-thymocyte globulin (ATG) followed by matched sibling donor hematopoietic cell transplantation (HCT) in patients with Fanconi Anemia (FA).

## 2.0 OBJECTIVES

### 2.1 Primary Objective

To determine the probability of engraftment in FA patients treated with CY, FLU and ATG followed by an HLA-genotypically identical related donor HCT.

### 2.2 Secondary Objectives

To evaluate the incidence of:

2.2.1 acute (aGVHD) and chronic (cGVHD) graft-versus-host disease
2.2.2 regimen-related toxicity (RRT) based on transplant outcomes through day 100
2.2.3 one year survival
2.2.4 late secondary malignancies (e.g. squamous cell carcinoma of head and neck, and cervix)

## 3.0 STUDY DESIGN

This is a study designed to evaluate neutrophil engraftment in Fanconi anemia (FA) patients transplanted with CD34+ selected bone marrow stem cells after a chemotherapy regimen consisting of CY, FLU and ATG.

## 4.0 BACKGROUND AND RATIONALE

HCT from an allogeneic donor is the only treatment with curative potential for patients with the hematological complications of FA. Durable engraftment and long term survival has been observed in matched sibling donor HCT after preparative regimens consisting of limited field irradiation (LFI) and low dose cyclophosphamide (CY).[1-7]

For the IBMTR, Gluckman et al. (1995) analyzed the outcome of 151 patients who received HCT for FA from HLA-identical siblings.[5] Age at transplant ranged from 1 to 39 years (median 10 years). Preparative therapies consisted of low dose CY (15 to 25 mg/kg) and limited field irradiation (LFI; 400-1500 cGy) with or without ATG (n = 75), low dose CY and total body irradiation (TBI; 300-800 cGy) (n = 20), and high dose CY (≥100 mg/kg) with or without ATG (n = 25). GVHD prophylaxis regimens included cyclosporine (CSA) (n = 82), methotrexate (MTX) (n = 10), and (3) CSA and MTX (n = 28). Two year probability of myeloid engraftment was 92%. Probabilities of grade II-IV acute GVHD at day 100 was 42% and of chronic GVHD at 2 years 44%. Probability of survival at 2 years was 66%. In multivariate analysis, improved engraftment rates were observed with age of HCT ≤10 years, conditioning including ATG, and higher platelet count pretransplant. In multivariate analysis, decreased mortality was observed with younger age, use of ATG in the conditioning regimen, conditioning with LFI and low-dose CY versus ≥100 mg/kg CY and no irradiation, and the use of cyclosporin with or without methotrexate for GVHD prophylaxis.

Long-term follow-up studies indicates that although successful HCT may cure the hematological complications of FA, patients are still at high risk for malignancy with approximately 40% of patients developing a malignancy within 15-20 years after HCT.[8,9] In an analysis of 700 patients with FA (n=79) or aplastic anemia (n=621) treated with allogeneic HCT in Seattle or Paris, the Kaplan-Meier estimate developing any malignancy by 20 years after transplantation was 14%.[8] The probability of FA patients developing a malignancy by 20 years after HCT was 42%. For the entire cohort of patients, risk factors for developing malignancy identified in multivariate analysis included chronic GVHD and azathioprine therapy, and the diagnosis of FA. In the non-FA group, chronic GVHD and azathioprine therapy, or irradiation were associated with increased risk of malignancy.

The Paris group described the long-term follow-up of 50 FA patients transplanted from a related donor.[9] With a median follow-up of >6 years, 32 patients (64%) were alive with a probability of survival at 100 months of 58.5%. In multivariate analysis, the only factor significant for poor survival was receiving more than 20 transfusions prior to HCT. The most important long-term complication in this group of patients was the occurrence of epidermoid carcinoma. Six male patients developed epidermoid head and neck cancers. All six patients had extensive chronic GVHD. The mean elapsed time between transplantation and the diagnosis of cancer was 8.2 years (range 5-26 years). A seventh patient developed a liver carcinoma 16 years after HCT. This patient had an androgen-induced adenoma before HCT and chronic hepatitis C. The 8- and 13- year projected incidence of malignancy in this cohort of patients was 24% and 43% respectively.

In an attempt to reduce the risk of malignancy, the Seattle group used a preparative regimen of CY 120-200 mg/kg without irradiation in 41 FA patients receiving related donor HCT (MSD in 38).[10] GVHD prophylaxis was with CSA and MTX (n=27), CSA alone (n=6) or MTX alone (n=8). Durable engraftment was observed in 88% of evaluable patients. Two patients, each conditioned with 200 mg/kg CY developed squamous cell carcinoma of the oral cavity 9.4 and 11 years after transplant. One patient had been treated with azothrioprine for chronic GVHD however this medication had been discontinued 9 years before the diagnosis of the malignancy.

Smaller doses of CY have been used in a few cases. In Hammersmith, 3 FA patients were transplanted with HLA-identical sibling donor BM after a preparative regimen of CY 20 mg/kg in 2 cases and CY 80 mg/kg in one case.[11] GVHD prophylaxis consisted of CSA. All 3 patients achieved myeloid engraftment and long term survival. One patient developed grade II acute GHVD of the skin and gut.

To potentially decrease the long-term effects of HCT, we have chosen fludarabine (FLU) to replace the use of irradiation. FLU is an antimetabolite that has been shown to be an effective immunosuppressive agent in BMT conditioning therapy.[12-15] As FLU is not a cross-linking agent, it will likely be well tolerated in FA patients. The combination of FLU and CY may offer sufficient engraftment rates and decrease the risk of long term complications after HCT. There are a couple of published case reports from Israel using a fludarabine in FA patients.[16,17] In both cases, preparative therapy consisted of CY (10 mg/m$^2$), FLU (150 mg/m$^2$) and ATG (40-50 mg/kg). GVHD prophylaxis consisted of CSA. Stem cell sources were an HLA-matched cousin bone marrow in one case and an HLA-matched sibling umbilical cord blood in the second. Myeloid engraftment was achieved on day +12 in the bone marrow recipient and day +28 in the UCB recipient. Myeloid engraftment was achieved on day +28. Acute grade II GVHD of the gut and liver developed in the UCB recipient and was successfully treated with CSA and steroids.[16]

In summary, two factors appear to be associated with risk of late malignancy in FA patients: 1) TBI and 2) development of chronic GVHD. Therefore, in an attempt to decrease the risk of malignancy, we have developed a regimen that does not include irradiation and have incorporated TCD to reduce the risk of chronic GVHD. In view of the fact that we are the largest referral center for HCT for FA and we have considerable experience with TCD, we have the unique ability to evaluate this treatment plan. We anticipate transplanting 5 patients per year with sibling donors using this new protocol.

In April 2010, Isolex®300i System were no longer available as they ceased to be manufactured and therefore enrolment on arm 1 has been terminated. As of August 2010, CliniMACS Cell Selection System will be used for T cell depletion of all bone marrow products used in this protocol and recipients of BM will be enrolled on this new arm 3.

As of August 2010, 23 subjects have been enrolled on this protocol. All subjects achieved primary engraftment. One subject, a recipient of an HLA-matched maternal BM, developed secondary graft failure (had initially engrafted but later lost the graft). He was retransplanted using the same donor but later died from chronic GVHD. Two patients also died after transplant from relapsed MDS. In light of these events, in November 2002, the eligibility was changed to only allow for HLA-identical sibling donors and to exclude FA patients with MDS or acute leukemia. A fourth patient (age 43 years) died from an intracranial hemorrhage shortly after transplantation. No patient has developed acute or chronic GVHD after an initial transplantation. To date, 19 of 20 recipients of HLA matched sibling donor HCT who had aplastic anemia at the time of transplant are alive and well. This result is superior to any published report of matched sibling donor HCT in FA patients.

As of August 2010, we have successfully shown that neutrophil engraftment is achievable in FA patients transplanted with CD34+ selected bone marrow stem cells (using the Isolex®300i System) after a chemotherapy regimen consisting of CY, FLU and ATG (arm 1 of trial). As the Isolex®300i System is no longer available we want to open a new arm of this protocol to study whether neutrophil engraftment is achievable in FA patients transplanted with CD34+ selected bone marrow stem cells (using the CliniMACS Cell Selection System) after the same chemotherapy regimen consisting of CY, FLU and ATG (arm 3). We will continue to accrue patients in arm 2 of the study in which sibling donor UCB is used as the stem cell source. We will also continue to follow all patients for the development of acute and chronic GVHD and late effects.

In August 2012, arm 4 was added as it was recognized not all sibling donor bone marrow will require cell selection using the CliniMACS. The decision whether or not to do CD34 selection will be made on an individual patient basis. Enrollment plans were also updated. Using the August 2010 revision as the starting point, an additional 20 patients will be accrued.

## 5.0 ELIGIBILITY AND EXCLUSION CRITERIA/STUDY REGISTRATION

### 5.1 Inclusion Criteria

5.1.1 Patients must be <60 years of age with a diagnosis of FA.

5.1.2 Patients must have an HLA-A, B, DRB1 identical sibling donor. Related donors will be evaluated under the University of Minnesota donor protocol MT2012-14C. Patients and donors will be typed for HLA-A and B using serological or molecular techniques and for DRB1 using high resolution molecular typing. If a matched sibling marrow donor is not available, UCB may be used as a cell source. If unlicensed cord blood unit is used, patients will co-enroll on MT2011-13R: Infusion of Cell Populations from Unlicensed Umbilical Cord Blood Units.

5.1.3 Patients with FA must have moderately severe aplastic anemia (AA), early

myelodysplastic syndrome (MDS) with no excess blasts with or without chromosomal abnormalities.

5.1.3.1

1) platelet count <20 x $10^9$/L
2) ANC <5 x $10^8$/L
3) Hgb <8 g/dL

5.1.3.3 Early myelodysplastic syndrome, with multilineage dysplasia with < 5% blasts, with or without chromosomal anomalies.

5.1.4 Adequate major organ function including:
5.1.4.1 Cardiac: ejection fraction ≥45%
5.1.4.2 Hepatic: no clinical evidence of hepatic failure (e.g. coagulopathy, ascites)
5.1.4.3 Karnofsky performance status ≥70% or Lansky ≥50 (for patients < 16 years)

5.1.5 Women of child bearing age must be using adequate birth control and have a negative pregnancy test.

5.1.6 Written consent.

## 5.2 Exclusion Criteria

5.2.1 Active bacterial infection within one week of HCT.

5.2.2 Active fungal infection at time of HCT.

5.2.3 Late MDS with greater than 5% blasts in bone marrow.

5.2.4 AML or history of AML.

5.2.5 Malignant solid tumor (e.g. squamous cell carcinoma of the head/neck/cervix) within 2 years of HCT.

5.2.6 Pregnant or lactating female.

## 5.3 Patient Registration

To be eligible for registration to this study, the patient must meet each criteria listed on the eligibility checklist based on the eligibility assessment documented in the patient's medical record.

5.3.1 Registration with the Clinical Trials Office

Upon completion of the screening evaluation, eligibility confirmation and obtaining informed consent, a designated study staff person will enroll the patient into OnCore. At the time of registration, the patient's cell source/processing will be indicated in OnCore as one of the following study arms:

**Arm 1**: bone marrow processed using Isolex 300i (for patients enrolled through April 2010)
**Arm 2:** UCB – no processing. If one or both of the cord blood units used for the graft is unlicensed, the participant will co-enroll on University of Minnesota protocol MT2011-13R "Infusion of Cell Populations from Unlicensed Umbilical Cord Blood Units."
**Arm 3**: bone marrow processed using CliniMACS (for patients enrolled beginning with the August 2010 protocol version). Subjects enrolled on the November 1, 2016 version of the protocol will co-enroll on University of Minnesota Protocol MT2015-31 "CliniMACS CD34 Reagent System as a HUD for Obtaining CD34+ Cell-Enriched Products"
**Arm 4:** match sibling donor marrow without use of the CliniMACS

5.3.1 Patients Who Are Registered and Do Not Receive Study Treatment

If a patient is registered to the study, and is later found not able to begin the preparative

regimen (beginning with the first dose of fludarabine), for whatever reason, the patient will be removed from study and treated at the physician's discretion. The patient will be considered a screen/baseline failure and the reason for removal from study will be clearly indicated in OnCore.

## 6.0 TREATMENT SCHEMA

In order to provide optimal patient care and to account for individual medical conditions, investigator discretion may be used in the prescribing of all supportive care therapy (i.e. acetaminophen, diphenhydramine, G-CSF, antimicrobials, etc).

Patients will receive antifungal prophylaxis beginning 1 month prior to conditioning therapy, if possible. Refer to section 6.3.

See appendices IV and V for preparative and prophylaxis drug information including expected toxicities.

| Day | Treatment |
|---|---|
| -6 | CY 5 mg/kg IV<br>FLU 35 mg/m$^2$ IV<br>ATG 30 mg/kg/day IV<br>MP 2 mg/kg IV |
| -5 | CY 5 mg/kg IV<br>FLU 35 mg/m$^2$ IV<br>ATG 30 mg/kg/day IV<br>MP 2 mg/kg IV |
| -4 | CY 5 mg/kg IV<br>FLU 35 mg/m$^2$ IV<br>ATG 30 mg/kg/day IV<br>MP 2 mg/kg IV |
| -3 | CY 5 mg/kg IV<br>FLU 35 mg/m$^2$ IV<br>ATG 30 mg/kg/day IV<br>MP 2 mg/kg IV |
| -2 | FLU 35 mg/m$^2$ IV<br>ATG 30 mg/kg/day IV<br>MP 2 mg/kg IV |
| -1 | Rest day |
| 0 | HCT |
| +1 | Initiate G-CSF 5mcg/kg per day IV (continue until ANC $\geq$2.5 x $10^9$/L) |

The administration of the preparative regimen will follow the institutional dosing and supportive care guidelines. Dose and/or schedule adjustments consistent with the standard of care may be made on an individual patient basis as needed for safety.

### 6.1 Preparative Therapies

The preparative cytoreductive therapy will be identical for all patients. Toxicities related to

the preparative regimen are defined in Appendix IV.

6.1.1 Cyclophosphamide
CY 5 mg/kg is to be given as a 2 hour infusion with strict attention to vigorous hydration, fluid balance and maintenance of good urine output. Mesna (5 mg/kg) in divided doses may be given in the first 24 hours after CY.

6.1.2 Fludarabine
FLU 35 mg/m$^2$ will be given IV over 30 minutes. Preparation, administration and monitoring will be according to standard practice procedures.

6.1.3 Anti-thymocyte globulin
ATG (ATGAM; Upjohn Corporation) 30 mg/kg/day will be administered after MP on days -6, -5, -4, -3 and -2. ATG will be diluted in sterile normal saline or half normal saline for intravenous infusion to a concentration of 1-2 mg/mL and will be infused through the Hickman catheter over 4 - 6 hours. In the event of a significant reaction to ATGAM, the agent will not be administered; instead patients will receive thymoglobulin (rabbit ATG) 1.5 mg/kg every 12 hours diluted in sterile normal saline or half normal saline for intravenous infusion to a concentration of 0.5 mg/mL and will be infused through the central venous line over 4 - 6 hours. In the event of a significant reaction to thymoglobulin, the agent will not be administered.

Methylprednisolone (MP) 2 mg/kg/day intravenously every 24 hours will be given from day -6 until day -2 as a premedication for ATG.

## 6.2 GVHD Prophylaxis

Patients will receive cyclosporine A (CSA) and mycophenylate mofetil (MMF) as GVHD prophylaxis.

### 6.2.1 CSA

Patients will receive CSA therapy beginning on day -3 with a taper commencing on day +100. For adults with normal baseline renal function (creatinine <1.2 mg/dL), the initial dose will be 2.5 mg/kg IV over 2 hours every 12 hours; for children <40 kg, the initial dose will be 2.5 mg/kg IV over 2 hours every 8 hours. CSA dosing will be monitored and altered as clinically appropriate per University of Minnesota Medical Center - Fairview pharmacy guidelines. Dose adjustments will be made on the basis of toxicity and low CSA levels with a trough level of <200 mg/L.

Once the patient can tolerate oral medications and has a normal gastrointestinal transit time, CSA will be converted to an oral form at a dose of 5.0 mg/kg PO every 12 hours. Beginning on day +100 or 1 month after control of GVHD (whichever is later), CSA will be tapered by 10%/week.

### 6.2.2 MMF

Patients will receive MMF therapy beginning on day -3 through day +30 or for 7 days after engraftment, whichever day is later, if no acute GVHD. Engraftment is defined as 1st day of 3 consecutive days of absolute neutrophil count [ANC] > 0.5 x $10^9$/L.

MMF will be given at a dose of 15 mg/kg/dose every 8 hours PO (to a maximum dose of 1 gram). The MMF dose may be adjusted to tablet sizes of 250 mg and 500 mg or administered using the suspension. MMF may be given IV at the same dose if PO not tolerated. MMF dosing is to be monitored and altered as clinically appropriate based on Pharmacist orders.

If the patient has acute GVHD requiring systemic therapy, MMF may be stopped 7 days after initiation of systemic therapy for acute GVHD (e.g. resolution of skin rash, vomiting, and diarrhea).

### 6.3 Antifungal Therapy

Patients will receive anti-fungal prophylactic therapy with itraconazole, posoconazole, caspofungin or micofungin beginning up to 1 month prior to conditioning therapy. Antifungal prophylaxis will be given until at least 100 days after HSCT.

### 6.4 Stem Cell Infusion (day 0)

Bone marrow and UCB grafts will be evaluated for sterility, cell viability, nucleated cell count, CD34 and CD3 counts.

6.4.1 Bone Marrow (arm 1 and 3)
Refer to Section 7 for bone marrow collection and processing information. Related donors will be evaluated and collected per MT2012-14C. Infuse per University of Minnesota institutional guidelines for bone marrow.

6.4.2 Umbilical Cord Blood (arm 2)
Selection of UCB will be based upon institutional guidelines. The selection of the UCB unit will be based on level of HLA match and cell doses following a University of Minnesota specific UCB Selection Algorithm. The ideal body weight will be determined for all patients.

The UCB will not be manipulated. If one or both of the cord blood units used for the graft is unlicensed, the participant will co-enroll on University of Minnesota protocol MT2011-13R "Infusion of Cell Populations from Unlicensed Umbilical Cord Blood Units."

The hydration regimen will be given following institutional guidelines. Patients should receive pre-medication acetaminophen and diphenhydramine hydrochloride 15 to 30 minutes before infusion.

After proper identification and crosschecking according to institutional guidelines, the UCB unit will be infused through a central line without in-line filtration per institutional guidelines.

### 6.5 G-CSF

Initiate G-CSF 5mcg/kg per day IV day +1 and continue until ANC $\geq 2.5 \times 10^9$/L.

## 7.0 HEMATOPOIETIC STEM CELL HARVEST AND PROCESSING

Donor marrow will be collected in the usual sterile manner. The harvested marrow (prior to TCD) should contain a minimum of $2.5 \times 10^8$ nucleated cells/kg recipient body weight with a goal of >5.0 x

$10^8$ nucleated cells/kg recipient body weight. The ideal body weight will be determined for all patients.

With the August 2010 protocol revision and the addition of study arm 3 (bone marrow processing using CliniMACS), CD34+ cells will be isolated from the bone marrow with the CliniMACS® Cell Selection System (Miltenyi Biotec). Each patient is to receive 1.0 x $10^5$/kg CD3+ cells. An add back infusion of CD3+ cells may be required to achieve this prescribed cell dose. Refer to the CMC for additional processing information and lot release criteria. With the August 2012 revision, arm 4 was added for bone marrow not using the CliniMACS. In August 2016, the University of Minnesota received approval to use the CliniMACS CD34 Reagent System as a Humanitarian Use Device (HUD) as protocol number MT2015-31. With the November 2016 amendment, we will be using MT2015-31 to co-enroll subjects needing CD34+ cell isolation.

Prior to activation of the August 2010 amendment, cell selection was done using the Isolex®300i System (Nexell Therapeutics, Inc.) under treatment arm 1 (bone marrow processing using Isolex®300i System).

## 8.0 REQUIRED OBSERVATIONS

### 8.1 Schedule of Activities

(See Appendix I)

### 8.2 Pre Study Screening Procedures

8.2.1 Chest radiograph and other radiographic studies (as clinically indicated)

8.2.2 High resolution CT of chest, abdomen and pelvis

8.2.3 Bone marrow aspirate and biopsy

8.2.4 Karnofsky or Lansky performance status (Appendix IV)

8.2.5 Ultrasound of liver and kidneys

8.2.6 Pulmonary function tests if capable (children >6 years, adults)

8.2.7 ECG; ECHO

8.2.8 Hematology with differential

8.2.9 Urinalysis and 24 hour urine creatinine clearance or GFR.

8.2.10 Pregnancy test (urine) - as clinically indicated

8.2.11 CMV Titer (donor/recipient)

### 8.3 Evaluation During Therapy Until Engraftment

8.3.1 CBC with platelet count daily until one week after ANC $\geq$ 5 x $10^8$/L, PLT $\geq$20 x $10^9$/L then twice weekly or as indicated

8.3.2 After stem cell infusion, GVHD evaluation weekly and as clinically indicated

### 8.4 Evaluation Post Engraftment To Discharge

8.4.1 Physical examinations weekly until discharge

8.4.2 CBC with platelet count weekly until discharge

8.4.3 GVHD evaluations as clinically indicated

8.4.4 Test peripheral blood and/or bone marrow for chimerism as clinically indicated

8.4.5 Review for events per section 9 and for the BMT database

### 8.5 Evaluation Post Engraftment at Day 60, 90-100 and 180 Then Once Yearly for 2 Years

8.5.1 CBC, differential and platelet

8.5.2 GVHD score

8.5.3 Physical exam

8.5.4 Peripheral blood Chimerism assay on day 21-30, 90-100, and 180 days, 1 and 2 years

8.5.5 Review for events per section 9 and for the BMT database

### 8.6 Engraftment Evaluation

Peripheral blood CBC counts will be monitored daily after transplantation until engraftment and weekly after engraftment while hospitalized. CBC counts will be evaluated at follow-up visits in the outpatient clinic. Chimerism assay of the blood will be sent on day 21-30, day 90-100, day 180, and 1 and 2 years post transplant.

## 9.0 EVENT REPORTING AND DATA AND SAFETY MONITORING PLAN

Toxicity and adverse events will be classified according to NCI's Common Terminology Criteria for Adverse Events V 4.0 (CTCAE). A copy of the CTCAE can be downloaded from the CTEP home page:
http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40

FOR UNLICENSED UCB UNITS ONLY: Selected expected adverse reactions determined to be caused by or at least possibly caused by the UCB units based on objective evidence will be reported in an expedited manner to the FDA under University of Minnesota IND BB-14797 (C. Brunstein, MD, PhD –sponsor/investigator).

FOR CD34+ SELECTED CELLS ONLY: Any clinically significant safety issues associated with cell processing failure or device malfunction regarding the CliniMACS® system must be reported to the PI (Dr. Margaret MacMillan) and Miltenyi (boston@miltenyibiotec.com) per institutional procedures under protocol MT2015-31.

Note: throughout this section the generic term "study drug" is used to refer to the study related treatment.

### 9.1 Adverse Event Reporting

#### 9.1.1 Definitions

The following definitions are based on the Code of Federal Regulations Title 21 Part 312.32 (21CFR312.32(a)).

**Adverse Event**: Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related.

**Suspected Adverse Reaction**: Any adverse event for which there is a reasonable possibility that the drug caused the adverse event.

**Life-Threatening Adverse Event Or Life-Threatening Suspected Adverse Reaction**: An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death.

**Serious Adverse Event Or Serious Suspected Adverse Reaction**: An adverse event or suspected adverse reaction is considered "serious" if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- Death

- A life-threatening adverse event
- 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 that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate 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.

**Unexpected adverse event or unexpected suspected adverse reaction**: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended. Thus, adverse events that occur as part of the disease process or underlying medical conditions are considered unexpected; however, they will not be reportable per section 9.1.2.

**Expedited (Rapid) Reporting**: Certain events may require rapid notification to entities providing patient safety oversight (e.g. IRB) as detailed in section 9.1.2.

**Stopping rule events**: The following events count toward a study stopping rule per section 10.5 and must be reported to the MCC Study Coordinator using the Early Stopping Rule Report Form found OnCore under the reports tab:
- graft failure at day +42
- death by day 100

Events that count toward an early stopping rule do not necessarily constitute a serious adverse event requiring expedited reporting and should be reported as such only if they meet the criteria for expedited reporting as defined in the above table.

In addition, the IRB considers any of the following problems/situations as meeting the definition of UPIRTSO:
- Any accidental or unintentional change to the IRB-approved protocol that increases risk or has the potential to recur
- Any deviation from the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject
- Any publication in the literature, safety monitoring report (including Data and Safety Monitoring Reports), interim result or other finding that indicates an unexpected change to the risk/benefit ratio of the research
- Any breach in confidentiality that may involve risk to the subject or others
- Any complaint of a subject that cannot be resolved by the research staff
- Any other possibly related event which in the opinion of the investigator constitutes an unanticipated risk

**FOR UNLICENSED UCB UNITS ONLY:** These patients will be co-enrolled on University of Minnesota protocol MT2011-13R. **Selected expected adverse reactions** determined to be caused by or probably caused by the UCB unit based on objective evidence will be reported in an expedited manner to the FDA under University of Minnesota IND BB-14797 (C. Brunstein, MD, PhD – sponsor/investigator). Included are the following:

- The unit is mislabeled or failure to pass local lot release
- Serious infusion reaction within first 24 hours after infusion
- Recipient bacteremia with clinical signs and symptoms related to a contaminated UCB within 24 hours after infusion

**FOR CD34+ SELECTED CELLS ONLY**: These patients will be co-enrolled on University of Minnesota protocol MT2015-31. Adverse events determined to be caused by or probably caused by the CD34+ enriched cell product for hematopoietic reconstitution based on objective evidence will be reported to the IRB and Miltenyi Biotech per institutional procedures under protocol MT2015-31.

### 9.1.2 Event Monitoring and Required Reporting

Patients will be monitored from the start of the preparative regimen through day 100 for events that are reportable according to the below table.

| Agency | Criteria for reporting | Timeframe | Form to Use | Submission address | Copy AE to: |
|---|---|---|---|---|---|
| **U of MN IRB** | Unanticipated death of a locally enrolled subject(s); New or increased risk; Any adverse event that require a change to the protocol or consent form – refer to the IRB website for complete details | 5 Working Days | IRB report form | irb@umn.edu | Masonic Cancer Center SAE Coordinator mccsaes@umn.edu |
| | Clinical deviations per current IRB reporting requirements | | OnCore Deviation Form | | |
| **MCC SAE Coordinator** | Any event that counts toward a study stopping rule (refer to section 10.3) | At time of reporting | Event Form | SAE Coordinator mccsaes@umn.edu | Not applicable |

*downloadable from http://www.research.umn.edu/irb/forms.html

The SAE Coordinator will provide the Masonic Cancer Center's Data and Safety Monitoring Council (DSMC) with the SAE in an appropriate format depending on the individual SAE (as reported or in a summary format).

## 9.2 Study Data Collection and Data and Safety Monitoring Plan

### 9.2.1 Data Collection

Specific transplant related endpoints will be recorded in the University of Minnesota Blood and Bone Marrow Database as part of the historical database maintained by the department.

### 9.2.2 Data and Safety Monitoring Plan

The study's Data and Safety Monitoring Plan will be in compliance with the University of Minnesota Masonic Cancer Center's Data & Safety Monitoring Plan (DSMP), which can be accessed at http://z.umn.edu/dmsp .

For the purposes of data and safety monitoring, this study is classified as moderate risk. Therefore the following requirements will be fulfilled:

- The Masonic Cancer Center Data and Safety Monitoring Council (DSMC) will review the trial's progress twice yearly
- The PI will comply with at least twice yearly monitoring of the project by the Masonic Cancer Center monitoring services.
- The PI will oversee the submission of all reportable adverse events per the definition of reportable in section 9.1.2 to the Masonic Cancer Center's SAE Coordinator and the University of Minnesota IRB.

In addition, at the time of the continuing review with the University of Minnesota IRB, a copy of the report with any attachments will be submitted to the Cancer Protocol Review Committee (CPRC).

9.2.3 Record Retention

The investigator will retain study records including source data, copies of case report form, consent forms, HIPAA authorizations, and all study correspondence in a secured facility for at 6 years after the study file is closed with the IRB.

In addition, the Clinical Trials Office (CTO) will keep a master log of all patients participating in the study with sufficient information to allow retrieval of the medical records for that patient.

Please contact the CTO before destroying any study related records.

## 10.0 EXPERIMENTAL DESIGN AND STATISTICAL CONSIDERATIONS

Recent experience has demonstrated that among patients using a stem cell source from processed bone marrow using Isolex 300i, the primary graft failure rate is 0%. Therefore we expect that graft failure will be observed in very few patients.

### 10.1Statistical Endpoints

10.1.1 Primary Endpoint

The proportion of patients with primary neutrophil engraftment by day 42 post-transplant

10.1.2 Secondary Endpoints

- The cumulative incidence of acute and chronic GvHD
- The cumulative incidence of regimen-related toxicity based on transplant related outcomes by day 100 post-transplant
- Probability of survival by one year post-transplant
- The proportion of late second malignancies

## 10.2 Statistical Analysis

Simple proportions along with 95% confidence intervals will be used to evaluate the incidence of primary neutrophil engraftment. Medians and ranges for time to engraftment will also be given. A competing risk of early death is not expected among these patients. Cumulative incidence will be used to estimate regimen-related toxicity and GvHD; treating non-event deaths as competing risks. Kaplan-Meier curves will be used to estimate overall survival. Additional descriptive statistics will be used for safety parameters and to evaluate data from the UCB cohort.

## 10.3 Sample Size Justification

The maximum sample size will be 39 patients (29 for arm 3 using bone marrow processing using CliniMACS and 10 or fewer patients receiving UCB). Twenty-nine patients are sufficient to maintain an overall type I error of 5% while providing 80% statistical power. The sample size calculation is based on the Simon method using a minimax two-stage design (Simon, R [1989], Optimal two-stage designs for phase II clinical trials. Controlled Clinical Trials, 10; 1-10).

The two-stage design uses a two-step enrollment procedure with early stopping if less than 80% of patients fail to engraft. More than 95% engraftment shows evidence that this procedure is worthy of further investigation.

The first enrollment will include 9 patients with an early stop if 7 or fewer patients engraft. If 8 or more engraft, the study will continue to enroll patients until 29 patients are enrolled.

After 29 patients are enrolled, the regimen will not be considered worthy of further study if 26 or fewer patients engraft. If 27 or more engraft, this regimen will be considered worthy of further study.

## 10.4 Enrollment Plan

An additional 20 patients will be enrolled on this study beginning with the August 2012 version.

## 10.5 Monitoring Guidelines: Stopping Rules

Based on the enrollment of 20 additional patients beginning with the August 2012 version of the protocol, the stopping rules are as follows:

**Day 100 Mortality**

Mortality will be continuously monitored so that the study will stop and the protocol will be re-evaluated if early mortality exceeds the boundary. Monitoring will take place separately for both arms. Given a hypothesized mortality rate of 30%, a maximum tolerated rate of 50% and a maximum sample size of 20 patients, the trial will be stopped and reviewed if: 6/6, 7/9, 8/11, 9/14, 10/16, or 11/19 patients die prior to day 100. This has a type I error rate of 5% and a power of 80%.

**Graft failure By Day 42**

Graft Failure will be continuously monitored so that the study will stop and the protocol will be re-evaluated if graft failure exceeds the boundary. Monitoring will take place separately for both arms. Given a hypothesized graft failure rate of 10%, a maximum tolerated rate of 20% and a maximum sample size of 20 patients, the trial will be stopped and reviewed if: 4/4, 5/10, 6/17, or 7 patients have graft failure. This has a type I error rate of 5% and a power of 80%.

# 11.0 CONDUCT OF THE STUDY

## 11.1 Ethical Considerations

While there will be every effort to seek out and include females and minority patients, the patient population is dependent upon the referral pattern and the ability to locate a matched sibling donor or a UCB unit. Females and minority patients are eligible for all aspects of the study and their participation will be actively encouraged.

## 11.2 Good Clinical Practice

The study will be conducted in accordance with the appropriate regulatory requirement(s). Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

## 11.3 Informed Consent

All potential study participants will be given a copy of the IRB-approved consent to review. The investigator or designee will explain all aspects of the study in lay language and answer all questions regarding the study. If the participant decides to participate in the study, she/he will be asked to sign and date the consent document. Patients who refuse to participate or who withdraw from the study will be treated without prejudice.

## 12.0 REFERENCES

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## APPENDIX I - SCHEDULE OF ACTIVITIES FOR EACH PATIENT

Scheduled evaluations prior to engraftment (day 30) may be performed +/-3 days from the targeted date; assessments to be performed between engraftment and day 100 may be done +/-7 days of the targeted date; assessments after day 100 may be performed +/-30 days of the targeted date. In addition, targeted days may be altered as clinically appropriate.

| Activity | Pre-BMT | Day +1 to Engraftment | Post-engraftment to Discharge (days <30 minimum) | Short-term Post-engraftment Follow-up (days 31-100 minimum) | Long-term Post-engraftment Follow-up (>day 100) |
|---|---|---|---|---|---|
| Informed consent | x(admission) | | | | |
| Clinical evaluation | | | | | |
| Karnofsky/Lansky score | x(admission) | x(2) | x(2) | x(2, discharge home) | x(day 180, 360, , 720) |
| CXR | x(admission) | | | | |
| PFT (> 6 years of age) | x(admission) | | | | x(3) |
| ECG/ECHO | x(admission) | | | | |
| US liver & kidneys | x(admission) | | | | |
| CT chest, abdomen, pelvis | x(admission) | | | | |
| Cr/Cr clearance | x(admission) | | | | |
| Laboratory evaluation | | | | | |
| CBC/differential | x(admission) | x(1) | x(1) | x(2) | x(day 180, 360, , 720) |
| Blood chimerism | $PBL_{patient}$ for DNA<br>$BM_{donor}$ for DNA | | x(3) | x(day 90-100) | x(day 180, 360, 720) |
| Monitoring for reportable events and stopping rules (section 9.1.2) | | x | x (day 30) | x(day 60, 90-100) | |
| GVHD evaluation | | x(1) | x(1) | x(3) | x(3) |

x(1)=perform test daily
x(2)=perform test weekly
x(3)=perform test as clinically indicated
x(day)=perform test on day indicated

## APPENDIX II - GRAFT VERSUS HOST DISEASE

Patients will be considered evaluable if they demonstrate donor cell engraftment and survive to day 42. Organ involvement will be staged using the criteria outlined in the table below. Biopsy of each organ site at diagnosis or major change in disease activity will be performed unless clinical circumstances make it impossible.

### Grading for Treatment Criteria

a. Mild GVHD = Skin stage I-II only (Equivalent to Seattle Grade I).

b. Moderate GVHD = Skin stage I-III and/or liver I-IV and/or Gastrointestinal tract (GI) I-III and/or Upper GI (UGI). (Equivalent to Seattle Grade II, III).

c. Severe GVHD = Any stage IV along with severe clinical illness.

Patients progressing during initial therapy or not improving sufficiently after 2 courses of therapy are to be treated as severe GVHD.

Criteria for staging acute graft-versus-host disease.

| Stage | Skin | Liver | GI Tract |
|---|---|---|---|
| I | Maculopapular rash <25% of body surface | T Bili 2-3 mg/dL or T Bili <2 mg/L with 5'nuc or alk phos 2 x NL and liver bx consistent with GVHD | >500 mL diarrhea/day (300 mL/$m^2$/day+); Nausea; anorexia |
| II | Maculopapular rash 25-50% of body surface | T. Bili 3-6 mg/dL | 1000 mL diarrhea/day (>600 mL/$m^2$/day+); Vomiting; food intolerance |
| III | Generalized erythroderma | T. Bili 6-15 mg/dL | >1500 mL diarrhea/day (>1000 mL/$m^2$/d+) |
| IV | Generalized erythroderma with bullous formation and desquamation | T. Bili >15 mg/dL | Severe abdominal pain with or without ileus |

## APPENDIX III - PERFORMANCE STATUS SCALE

## KARNOFSKY PERFORMANCE STATUS SCALE

| Percentage | |
|---|---|
| 100 | Normal, no complaints, no evidence of disease |
| 90 | Able to carry on normal activity; minor signs or symptoms of disease |
| 80 | Normal activity with effort; some signs or symptoms of disease |
| 70 | Cares for self; unable to carry on normal activity or do active work |
| 60 | Requires occasional assistance, but is able to care for most of his/her needs |
| 50 | Requires considerable assistance and frequent medical care |
| 40 | Disabled; requires special care and assistance |
| 30 | Severely disabled, hospitalization indicated. Death not imminent |
| 20 | Very sick, hospitalization necessary, active supportive treatment necessary |
| 10 | Moribund, fatal processes, progressing rapidly |
| 0 | Dead |

Reference

Karnofsky DA. Editorial: Meaningful clinical classification of therapeutic responses to anti-cancer drugs. Clin Pharmacol Ther 2:709-712, 1961.

## LANSKY PLAY PERFORMANCE STATUS SCALE

| Percentage | |
|---|---|
| 100 | Fully active, normal |
| 90 | Minor restrictions in physically strenuous activity |
| 80 | Active, but tires more quickly |
| 70 | Both greater restriction of, and less time spent in, play activities |
| 60 | Up and around, but minimal active play; keeps busy with quieter activities |
| 50 | Gets dressed but lies around much of the day, no active play; able to participate in all quiet play and activities |
| 40 | Mostly in bed; participates in quiet activities |
| 30 | In bed; needs assistance even for quiet play |
| 20 | Often sleeping; play entirely limited to very passive activities |
| 10 | Unresponsive |
| 0 | Dead |

REFERENCE

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## APPENDIX IV - EXPECTED TOXICITIES

A. Toxicities associated with the preparative therapies:

**Cyclophosphamide**

| Common<br>Occurs in 21-100 people out of 100 | Less Frequent<br>Occurs in 5-20 people out of every 100 | Uncommon<br>Occurs in <5 people out of every 100 |
|---|---|---|
| hypotension<br>nausea and vomiting<br>diarrhea<br>hemorrhagic cystitis<br>leukopenia<br>amenorrhea | | syndrome of inappropriate ADH<br>hemorrhagic myocardopathy<br>Stevens-Johnson syndrome<br>toxic epidermal necrolysis<br>azoospermia<br>oligozoospermia<br>interstitial pneumonia |

Adequate diuresis must be maintained before and after Cyclophosphamide (CY) administration to help prevent hemorrhagic cystitis. Hemorrhagic cystitis is a serious and potentially life-threatening toxicity of Cytoxan therapy. The incidence varies but has a relationship to dose. The toxicity is related to the direct contact of CY metabolites with the uroepithelium. The recommended maintenance fluid is 3000 cc/$m^2$/24 hours. This rate should be started at least six hours before the CY dose. Bolus furosemide can be administered to maintain urine output. Patients should be weighed bid from day -2 to day 0 of HCT. High urine volume with frequent voiding (q 1-2 hour) is important to decrease the toxic exposure. Mesna (total 60 mg/kg/day in 5 divided doses) may be given before CY, and 3, 6, 9 and 12 hours after CY to reduce the risk of hemorrhagic cystitis.

CY has an antidiuretic effect that appears approximately 4 hours after and is maximal 10-14 hours after CY administration. This can be controlled with furosemide. In any patient with urine output inappropriate relative to the high volume IV input, furosemide should be given. It is critical to closely monitor electrolytes during this diuresis. See standing orders for administration of CY.

**Fludarabine**

| Common<br>Occurs in 21-100 people out of 100 | Less Frequent<br>Occurs in 5-20 people out of every 100 | Uncommon<br>Occurs in <5 people out of every 100 |
|---|---|---|
| severe suppression of blood counts<br>diarrhea<br>anorexia<br>mucositis<br>nausea/vomiting<br>stomatitis<br>osteoporosis<br>dysuria | chills<br>fever<br>GI bleeding<br>peripheral edema | neurotoxicity<br>agitation and confusion<br>blurred vision<br>peripheral neuropathy<br>hearing loss<br>headache<br>cerebellar syndrome<br>blindness<br>coma<br>weakness<br>depression<br>insomnia |

| Common<br>Occurs in 21-100 people out of 100 | Less Frequent<br>Occurs in 5-20 people out of every 100 | Uncommon<br>Occurs in <5 people out of every 100 |
|---|---|---|
| | | hemorrhagic cystitis (except in FA)<br>abnormal renal function test<br>autoimmune hemolytic anemia<br>deep venous thrombosis<br>aneurysms<br>pruritic skin rash<br>abnormal liver function/liver failure<br>constipation<br>transient ischemic attack<br>dysphagia<br>myalgia<br>arthralgia<br>renal failure |

**Thymoglobulin (ATG)**

| **Common** | **Less Frequent** | **Uncommon** |
|---|---|---|
| fever<br>chills<br>leukopenia<br>pain<br>headache<br>abdominal pain<br>diarrhea<br>hypertension<br>nausea<br>thrombocytopenia<br>peripheral edema<br>dyspnea<br>asthenia<br>hyperkalemia<br>tachycardia | malaise<br>dizziness | severe allergic reaction (anaphylaxis) |

Methylprednisolone (MP) - possible side effects include weight gain and water retention, puffiness of the face, high blood pressure, high blood sugar, bleeding from the stomach and intestines due to ulcers, and personality changes including depression. High blood pressure and water retention can be treated with medications and very high levels of blood sugar can be corrected with insulin.

Long-term use (which is not planned) has been associated with reversible muscle weakness and muscle thinning and cataracts. It has also been associated with bone thinning; this has sometimes progressed to areas of bone death, called "avascular necrosis" or "AVN", especially at the knees and hips. In addition, methylprednisolone or prednisone makes patients prone to infections.

B. Toxicities associated with T cell depletion of bone marrow

The toxicities and complications that could potentially result from the removal of lymphocytes from the donated bone marrow are failure of bone marrow engraftment (due to loss or damage to hematopoietic progenitor cells), an increased risk of relapse, and an increased risk of EBV-associated lymphoproliferative syndrome.

C. Toxicities associated with Hematopoietic Stem Cell Transplantation

- nausea and vomiting
- possible allergic reaction (including itching, hives, flushing [red face], shortness of breath, wheezing, chest tightness, skin rash, fever, chills, stiff muscles, or trouble breathing)
- graft-versus-host-disease (GVHD)
- veno-occlusive disease,
- mucositis,
- infections (sepsis)

D. Toxicities associated with GVHD Prophylaxis

| **Cyclosporine (CSA)** | **Mycophenolate Mofetil (MMF)** |
|---|---|
| nephrotoxcity | pancytopenia |
| seizures | headache |
| hypertension | insomnia |
| hirsutism | electrolyte imbalances |
| increased risk of relapse | leg cramps/bone pain |
| thrombotic thrombocytopenic purpura | hypertension |
| electrolyte imbalances | dizziness |
| paresthesias/neuropathy | hyperglycemia |
| gingival hyperplasia | rash |
| increased risk of opportunistic infection | nausea/diarrhea |

.E. G-CSF

In general, this drug has few serious side effects. Side effects include fever, feeling tired, bone pain, and enlargement of the spleen. Additionally, there is a rare risk of allergic reaction to this drug.

# APPENDIX V – DRUG FORMULATION AND AVAILABILITY

**Cyclophosphamide**
Cyclophosphamide is a nitrogen mustard-derivative, polyfunctional alkylating agent. Cyclophosphamide for injection is commercially available as a sterile solution in sodium chloride or as a powder that is reconstituted in sterile or bacteriostatic water. Institutional guidelines for handling, reconstitution and administration should be followed. Cyclophosphamide can cause temporary hair loss, nausea, vomiting, diarrhea, and lowering of the blood cell counts or hemorrhagic cystitis. Anti-emetics should minimize nausea and vomiting. At the dose of cyclophosphamide used in this study, these symptoms are expected to be modest in severity.
**Formulation**: Parenteral 100 mg and 500 mg vials. Commercially available.
**Storage**: Room temperature.
**Mixing**: I.V. drug should be mixed with sterile water for I.V. use. Dissolves very slowly with cold water. It helps to warm the water or to warm the vial after adding water.
**Stability**: I.V. solution should be used within 12 hours of mixing.
**Administration**: Cyclophosphamide is added to D5W and administered I.V.

**Fludarabine**
Fludarabine is an antimetabolite. Fludarabine for injection is commercially available as a lyophilized cake that is reconstituted in sterile water. Institutional guidelines for handling, reconstitution and administration should be followed. Fludarabine can cause lowering of blood counts, suppression of the immune system, nausea and vomiting, fever, hypersensitivity reaction, tumor lysis, transient elevation in serum transaminases, and neurotoxicity at doses generally higher than administered in this study.
**Formulation**: 50 mg vial as a white lyophilized cake for IV use. Commercially available.
**Storage**: Room temperature.
**Mixing**: Add 2 mL sterile water to vial to give a final concentration of 25 mg/mL.
**Stability:** I.V. solution should be used within 8 hours of mixing.
**Administration:** Fludarabine is further diluted in 100 mL of 5%dextrose or 0.9% sodium chloride

**Thymoglobulin (anti-thymocyte globulin)**
Thymoglobulin (Genzyme) is a purified pasteurized, gamma immune globulin obtained by immunization of rabbits with human thymocytes. This immunosuppressive product contains cytotoxic antibodies directed against antigens expressed on human T-lymphocytes. Institutional guidelines for handling, reconstitution and administration should be followed. Common toxicities include fever, chills, leukopenia, pain, headache and nausea.
**Formulation:** Intravenous Lyophilized Powder
**Storage:** Store in refrigerator between +2 degrees C to +8 degrees C, protect from light, do not freeze
**Mixing:** 5-ml sterile water for injection as diluent.
**Stability:** Should be used within 4 hours of reconstitution if kept at room temperature.
**Administration:** Infusion – deliver Thymoglobulin doses per current University of Minnesota BMT unit horse ATG infusion procedures.

**Methylprednisolone (MP)**
**Availability**: Commercially available.
**Storage**: Store at controlled room temperature 20° to 25°C (68° to 77°F)
**Administration:** MP will be administered at the dose of 2 mg/kg/day intravenously every 24 hours day -6 until day -2 as a premedication for ATG. MP may be given as an IV push.

**Cyclosporin A (CSA)**
**Availability**: Commercially available.
**Storage**: Store the unmixed drug and oral formulation at room temperature below 86 degrees F (30 degrees C) away from heat and light.
**Stability:** I.V. solution should be used within 24 hours of mixing.
**Administration:** Cyclosporin A (CSA) will start day -3 per section 6.2.1. CSA dosing will be monitored and altered as clinically appropriate per University of Minnesota Medical Center - Fairview pharmacy guidelines. Dose adjustments will be made on the basis of toxicity and/or low CSA levels.

**Mycophenolate Mofetil (MMF)**
**Availability**: Commercially available.
**Storage**: Store powder and reconstituted/infusion solutions at 25°C (77°F); excursions permitted to 15° to 30°C (59° to 86°F).
**Administration:** MMF will start day -3 MMF therapy beginning on day -3 through day +30 or for 7 days after engraftment, whichever day is later, if no acute GVHD per section 6.6.2.

**G-CSF**
**Availability**: Commercially available.
**Storage**: G-CSF should be stored in the refrigerator at 2° to 8°C (36° to 46°F). Avoid shaking. Prior to injection, NEUPOGEN® may be allowed to reach room temperature for a maximum of 24 hours. Any vial or prefilled syringe left at room temperature for greater than 24 hours should be discarded.
**Administration:** Initiate G-CSF 5mcg/kg per day IV day +1 and continue until ANC $\geq 2.5 \times 10^9$/L.