UNIVERSITY OF MINNESOTA BONE MARROW TRANSPLANTATION PROGRAM

ALLOGENEIC BONE MARROW TRANSPLANTATION USING LESS INTENSIVE THERAPY

MT 2001-10
CPRC # 2001LS058

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PROTOCOL CHANGES

DATE	CHANGE
11/12/2013	Add appendix III and create a separate consent form as a mechanism to enroll 10 patients using a related donor from select diseases (AML, ALL, CML. HL, NHL, and MDS) to act as controls for MT2012-06 - Inducible Regulatory T Cells (iTreg) study Section 3 – add a paragraph regarding optional sub-study Update Section 9.3 to current DSMC reporting requirements and new email address
03/21/2011	Update to current Cancer Center and IRB format – remove references to MT2004-03 as study has closed to enrollment; update MMF dose to 1.5 grams
03/08/2010	3.1.1 and 3.1.2: Age limit changed to 75 years or less 5.12 Starting date of G-CSF post transplant changed to Day +5
10/12/09	2.0 Background and Rationale: Updated data regarding fludarabine dosing changes and interim analysis of MT 2001-10 subjects enrolled to date added 3.2.6 Updates to MDS disease criteria 3.2.7 Remove breast cancer as a disease entity treated by allogeneic transplant 3.5.8 Updated Myeloma disease response exclusion criteria 5.0 Treatment Schema: Fludarabine dose changed to 30mg/m2 daily 13.1 and 13.2: Updates to statistical analysis and stopping rules to include expansion to enroll an additional 150 patients.
6/18/2009	Dr. Erica Warlick replaces Dr. Marcie Tomblyn as PI
4/22/2009	Added an arm to co-enroll subjects from MT2004-03;
2/24/2006	Data and Safety Monitoring plan updated to reflect phase 2 status of study (section 13.5).
6/10/2005	Section 4.0 (Donor Eligibility) If donor is unable to undergo apheresis, marrow harvest will be acceptable. Section 4.2. Clarified that the target dose is for both unrelated and related donors. Section 13.2 (stopping rules). Clarified that if any of the stopping rules in arms 1-6 are reached, the study will be stopped
2/8/2005	Dr. Marcie Tomblyn takes over as Principal Investigator
11/12/2003	--Clarified that either marrow or PBSC will be transplanted (section 3.1, 5.6) --Added Chronic myeloprolifterative disorder to the eligibility --Replaced Anne Goldman, Ph.D. with Todd Defor, M.S., on the study committee.
1/30/2004	Section 3 Eligibility – minor edits for clarification and consistency throughout protocols; Metastic Melanoma and Small Cell lung cancer removed from list of eligible diseases Section 5.3 GVHD prophylaxis revised for consistency with program policy Section 6 Required observations. Blood draw for RFLP day 35 removed; KLH and tetanus vaccines removed. Section 8.0 Reporting of adverse events. Revised to be consistent with program policy Section 9.1.1 DLI eligibility revised for clarity Section 12 Endpoints. Minor revisions to clarify post transplant clinical evaluation dates Section 13 Experimental Design and Statistical considerations section revised to include an increase in target subjects to 80 (and rationale); added Data Safety and monitoring plan.
10/28/04	Section 3.2.9 Eligibility. Added Marrow Aplasia (as a consequence of AHN-12 therapy) to the eligibility of this study.
11/16/2004	Introduction of a new arm to this study labeled Arm 7 which will cater to high-risk patients transplanted with marrow aplasia after chemotherapy or radio-labeled antibody. Separate stopping rules have been added to the protocol to allow for appropriate patient monitoring and safety of these patients. Standard risk patients will continue to be transplanted as previously on and have been labeled as Arms 1-6 by our database. The high risk patients will be analyzed as Arm 7. • As we have recently modified the way we select unrelated volunteer donors (must be 7-8/8 allele HLA-match unless the patient less than 18 years), the wording of the graft selection section has been changed accordingly. • The definition of leukemic remission for standard risk patients has been clarified to resolve some confusion for borderline patients as to whether they are eligible or not. • Adequate organ function has been further defined to reflect the necessity for satisfactory renal function to be transplanted on this protocol safely. • Patients that are severely malnourished have been excluded from the protocol due to the risk of high

DATE	CHANGE
	transplant related mortality in this group. • High grade lymphomas or Hodgkin's disease that are progressive on salvage therapy have been excluded as these patients are unlikely to benefit from the transplant procedure. • Clarification of the current accepted practice re dosing of G-CSF for donors has been made and volunteer donors can give a product consisting of either BM or PB. • Which patients require the addition of ATG to their conditioning regimen has been clarified. • The days that the ATG is administered have been changed from days -3, 2, 1 to days -6, 5, 4. • Clarification of the cyclosporine and mycophenolate dosing to insure the wording in the protocol is consistent with current accepted clinical practice. • In an effort to identify occult fungal infection in patients at high risk for this complication pre-transplant a mandated chest CT has been added to the work-up studies for high-risk patients. • Clarification of the wording of who should receive donor lymphocyte infusions so its consistent with current accepted practice has been included. • The statistical section has been modified appropriately to incorporate the new stopping rules for the high-risk patients now labeled as Arm 7.

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1 PURPOSE

This treatment protocol will study the efficacy of a nonmyeloablative preparative regimen consisting of fludarabine, cyclophosphamide, and low dose TBI to promote engraftment after allogeneic hematopoietic stem cell transplant. Based on the initial favorable outcomes with day 100 treatment related mortality far below the stopping rule of 30%, the study was amended in October 2009 to enroll an additional 150 patients with a lower fludarabine dose of 30mg/m^2 Day -6 through -2.

Objectives:

- To evaluate the nonmyeloablative regimen for prompt and durable donor engraftment.
- To determine if nonmyeloablative transplantation using this regimen has an acceptable safety profile
- To evaluate the risks of Graft versus Host Disease (GVHD)
- To evaluate the anti-neoplastic potency of nonmyeloablative hematopoietic stem cell transplantation
- To evaluate the effect of lower doses of daily fludarabine on treatment related mortality (TRM)

2 BACKGROUND AND RATIONALE

High dose conditioning regimens followed by allogeneic bone marrow transplantation can provide durable remissions in a number of malignant diseases. The efficacy of such therapy is partly due to the conditioning and partly due to the immunological effect of the graft on the malignant cells of the recipient, the graft-versus-malignancy effect (GVM). However, such therapy is suitable only for a minority of patients due to the toxicity of the high dose preparative regimens and time of pancytopenia accompanying traditional transplantation. Thus, older patients and patients with certain end organ toxicity and/or co-morbidities are not eligible for such therapy.

It was previously thought that intensive marrow ablative regimens were required to establish allogeneic engraftment. Several recent studies have shown that that is not the case [1-6]. These early reports of nonmyeloablitive or "mini" transplants suggest that immunosuppression without myeloablation can be associated with allogeneic engraftment and that these regimens are less toxic. This allows investigation of the GVM effect in patients not previously eligible for transplantation.

Our goal is to find non-toxic regimens that provide sufficient immunosuppression for engraftment and that permit a graft versus malignancy (GVM) effect without increased GVHD [1,7,8]. Indeed we may think of this type of allogeneic transplant not as a "stem cell rescue" following ablative cancer therapy but primarily as an immunologic intervention [5,6,9-12]. Using a novel conditioning regimen based on fludarabine, cyclophosphamide and low dose TBI as a preparatory regimen may result in full donor chimerism. If mixed chimerism is not achieved, further manipulation of immunosuppressive medications and if necessary donor lymphocyte infusions can lead to complete chimerism with the potential for GVM effects [5,6].

The preceding study, MT1999-17, tested two moderate intensity, but still non-myeloablative (NMA) conditioning regimens for allotransplantation. This trial compared Buslfan, Cladribine and low dose total body irradiation to Busulfan, Fludarabine, and low dose total body irradiation. No unexpected

adverse events were seen. Engraftment was prompt although three graft failures in the Cladribine arm were observed out of 16 treated. As these results suggested no possibility that the Cladribine arm could be superior, and, in fact might be inferior, this arm was closed. The trial continued to enroll a total of 47 patients to date. Of these, 33 received sibling and 14 unrelated donor stem cells. Generally, they achieved prompt engraftment with only 3 of 47 having graft failure. Overall 26 of 47 patients survive, 7 of 14 receiving unrelated donor grafts and 19 of 33 receiving related donor transplants.

Because prolonged neutropenia and moderate mucositis was seen with Busulfan, Fludarabine and low dose total body irradiation despite the lesser intensity, in MT2001-10, a further modification in the conditioning regimen intensity is being investigated. A substitution of the busulfan with a single dose of cyclophosphamide (CY/FLU/TBI) was felt likely to be considerably less myelosuppressive. This has already been demonstrated in the sister protocol MT2000-15 (using umbilical cord blood) which was changed to CY/FLU/TBI in September 2001 (N=11).

Fludarabine has a very acceptable toxicity profile, with the major side effect being myelosuppression and immunosuppression [15]. Cyclophosphamide has been used in preparatory regimens for stem cell transplant for decades and the toxicity and side effect profiles are well known. As patients without prior combination chemotherapy are at greater risk for graft failure, those that have not had any combination chemotherapy in the six months prior to transplant will receive three days of ATG, the toxicity of which is relatively mild and well-known.

While fludarabine has shown a generally acceptable toxicity profile, dosing by creatinine clearance alone lacks ideal precision. In a separate study, independent work by Pamala Jacobson, Pharm D in the Deparment of Experimental and Clinical Pharmacology at the U of MN evaluated the role of fludarabine pharmacokinetic-pharmacodynamics in outcomes of engraftment, treatment related mortality (TRM), and overall survival (OS) in a subset of patients undergoing NMA allogeneic transplants.

Eighty-seven patients were evaluated and received the standard cytoxan 50mg/kg/day IV Day -6, plus fludarabine 40mg/m2/d IV days -6 to -2 and TBI 200cGy on day -1 used in MT 2001-10. Pharmacokinetic sampling was performed after the first dose of fludarabine and analysis was completed using standard noncompartmental methods with calculation of area-under-the curve (AUC). The primary objective was to determine the relationship between fludarabine metabolite (F-ara-A) exposure and neutrophil engraftment with secondary objectives evaluating the relationship between F-ara-A exposure and incidence of GVHD, TRM, and OS. The results revealed no impact of F-ara-A pharmacokinetic parameters on engraftment. Rates of grade II-IV aGVHD were lower in patients with an AUC >6.5 ug*hr/ml at 14% (95% CI: 0-32%) versus 52% (95% CI: 39-65%) in those with AUC < 6.5 ug*hr/ml (p=0.04). TRM was also significantly impacted by F-ara-A concentrations. Overall TRM was 21% at 6 months (95% CI, 12-29%). However, in univariate analysis, patients with F-ara-A AUC > 6.5 ug*hr/ml had 6 month TRM of 50% (95% CI: 23-77%) while those with an AUC < 6.5 ug*hr/ml had a TRM of 15% (95% CI: 7-23). In multivariate analysis, the only independent predictor of TRM was F-ara-A AUC > 6.5 ug*hr/ml with a RR of 4.56 (95% CI:1.22-17.12) (p=0.02). Overall survival was also impacted by higher F-ara-A AUC. Within this study, those patients with renal dysfunction as measured by creatinine clearance estimates, had fludarabine dose reduction of 20-25%. Despite this, these patients still had higher plasma

concentrations then expected, had reduced clearance of F-ara-A and prolonged drug half-life. (Data not yet published) Given the data of higher TRM seen with higher F-ara-A AUC, we will be adjusting the daily fludarabine dosing from 40mg/m2 daily Day -6 through -2 to 30mg/m2 daily Day -6 through -2. Study regarding possible individualized patient specific fludarabine dosing will likely be incorporated into future trial amendments in order to provide a dosing approach superior to creatinine clearance calculations.

Cyclosporine A (CsA) and MMF (mycophenolate mofetil) will be used exactly as in 1999-17. CsA inhibits T cell lymphokine production, but is not effective as a single agent [16,17]. MMF is an inhibitor of purine nucleoside metabolism and has been shown to provide an anti-GVHD effect as a single agent and has synergistic anti-GVHD effect when combined with CsA in dogs [18].

In preparation for upcoming renewal of MT 2001-01, the outcomes of the first 123 patients enrolled on MT 2001-10 were analyzed. The median age was 57 (range 23-70) and median comorbidity score 3 (range 0-10). A median of 709 days elapsed from diagnosis to transplant (range 76-4614) and median follow-up of survivors was 919 days (range 100-2422). Sixty-five percent (n=80) of patients were male and 78% (n=96) had a Karnofsky performance status of ≥ 90 at time of transplant. Eighty-six percent (n=106) of patients had no prior HCT. Numerous hematologic malignancy diagnoses were transplanted under this protocol: AML (27%, n=33), NHL (28%, n=35), MDS (11%, n=12), Hodgkins (8%, n=10), multiple myeloma (8%, n=10), Other leukemia (8%, n=10), myeloproliferative diseases (5%, n=6), CML (3%, n=4), and ALL (2%, n=3). Fifty percent (n=61) of the patients had advanced disease at time of transplant.

All patients achieved engraftment by Day 42. Acute GVHD grades II-IV at 100 days developed in 37% of patients (95% CI, 28-46%). Severe aGVHD grades III-IV at 100 days developed in only 20% (95% CI: 13-27%) of patients. At 2 years, the incidence of cGVHD was 50% (95% CI: 41-60%). TRM at 100 Days was 14% (95% CI: 8-20%) and 1 year was 22% (95% CI, 14-29%). Four year overall survival (OS) for the entire group was 47% (95% CI: 37-57%). Disease subtype impacted 4 year OS: indolent NHL: 73% (95% CI: 28-93%), aggressive NHL: 59% (95% CI: 34-77%), and AML + MDS: 35% (95% CI: 19-52%). Progression free survival (PFS) at 4 years was 33% (95% CI: 24-43%) for the entire group. By disease subtype 4 year PFS was: indolent NHL: 73% (95% CI: 28-93%), aggressive NHL: 55% (95% CI: 32-73%), and AML + MDS: 31% (95% CI: 16-48%)(p=0.04). Relapse at 4 years was 32% (95% CI: 23-42%) for the entire group. Patients transplanted for AML + MDS had the highest relapse rates of 36% (95% CI 18 – 54%), those with aggressive NHL had relapse rates of 21% (95% CI, 3-40%) and those with indolent NHL rates of 0%.

Based on the above favorable outcomes thus far with MT 2001-10 with 100 Day TRM far below the stopping rule of 30%, we plan to enroll an additional 150 patients with the new, lower fludarabine dosing.

3 PATIENT/DONOR SELECTION AND REGISTRATION

Study entry is open to patients ≤ 75 years of age regardless of gender or ethnic background. While there will be every effort to seek out and include women and minority patients, the patient population is expected to be no different than that of other transplant studies at the University Of Minnesota.

Effective with the November 2013 revision adult patients (≥ 18 years) with a diagnosis of AML, ALL, CML, NHL, HL or MDS and who are using a related donor will be invited to participate in an optional sub-study to act as controls for MT2012-06 "Dose Escalation Study with Extension of Inducible Regulatory T Cells (iTregs) in Adult Patients Undergoing Non-Myeloablative HLA Identical Sibling Donor Peripheral Blood Stem Cell Transplantation." A separate consent form will request permission to collect additional blood for research related testing prior to transplant and at up to 7 time points over the 1st 100 days post-transplant. Once 10 patients have been successfully recruited and samples are collected, this sub-study will end. Please refer to appendix III for details.

Registration in OnCore will occur after the patient has signed the subject consent and eligibility is confirmed, but before any treatment has been administered.

3.1 Age and Graft criteria (all patients)

Patient's ≤ 75 years old with a 5/6 or 6/6 related donor match or a 7-8/8 HLA-A,B,C,DRB1 allele matched unrelated donor marrow and/or PBSC donor match

3.2 Disease Criteria (standard risk patients)

- **Acute myelogenous leukemia**— high risk CR1 (as evidenced by preceding MDS, high risk cytogenetics such as those associated with MDS or complex karyotype, or > 2 cycles to obtain CR); second or greater CR. Must be in remission by morphology. Patients in morphologic relapse/ persistent disease defined as > 5% blasts in normocellular bone marrow OR any % blasts if blasts have unique morphologic markers (eg auer rods) or associated cytogenetic markers that allows morphologic relapse to be distinguished are not eligible for arms 2 or 3. Note cytogenetic evidence of disease alone *without* morphologic relapse is acceptable. Also a small percentage of blasts that is equivocal between marrow regeneration vs early relapse is acceptable provided there are no associated cytogenetic markers consistent with relapse.
- **Acute lymphocytic leukemia**-- high risk CR1 as evidenced by high risk cytogenetics (eg t(9;22) or complex cytogenetic abnormalities) or > 1 cycle to obtain CR; second or greater CR. Must be in remission by morphology. Patients in morphologic relapse/ persistent disease defined as ≥ 5% blasts in normocellular bone marrow OR any % blasts if blasts have unique morphologic markers or associated cytogenetic markers that allows morphologic relapse to be distinguished are not eligible for arms 2 or 3. Note cytogenetic relapse or persistent disease *without* morphologic relapse is acceptable. Also a small percentage of blasts that is equivocal between marrow regeneration vs early relapse is acceptable provided there are no associated cytogenetic markers consistent with relapse.
- **Chronic myelogenous leukemia** all types except blast crisis (note treated blast crisis in chronic phase is eligible)
- **NHL, Hodgkins, chronic lymphocytic leukemia, multiple myeloma** demonstrating chemosensitive disease
- **Acquired bone marrow failure syndromes**

- **Myelodysplastic syndrome** of all subtypes including refractory anemia (RA) or all IPSS categories if severe pancytopenia, transfusion requirements not responsive to therapy, or high risk cytogenetics. Blasts must be less than 5%. If ≥5% requires therapy (induction or Hypomethylating agents) pre-transplant to decrease disease burden.
- **Renal cell cancer**
- **Chronic myeloproliferative disorder**, i.e. myelofibrosis

3.3 Disease Criteria (High Risk Patients)

Patients with refractory leukemia or MDS may be taken to transplant in aplasia after induction or re-induction chemotherapy or radiolabeled antibody.

3.4 Organ Function, Performance Status, Other Eligibility

3.4.1 Cardiac: No decompensated CHF, or uncontrolled arrhythmia; EF ≥ 35%
3.4.2 Pulmonary: DLCO ≥ 30% predicted, No O_2 Requirements
3.4.3 Liver: Transaminases < 5.0 x ULN; total bilirubin < 3 x ULN
3.4.4 Renal: Creatinine ≤ 2.0 mg/dl (adults) or creatinine clearance > 40 ml/min). All adults with a creatinine > 1.2 mg/dl or a history of renal dysfunction must have creatinine clearance (must be > 40 ml/min).
3.4.5 Albumin > 2.5 g/dL
3.4.6 Performance status - Karnofsky ≥ 60% (16 years and older) or Lansky ≥ 50 (less then 16 years of age).
3.4.7 If recent mold infection (e.g. aspergillus) must have minimum of 30 days of therapy and responsive disease and be cleared by Infectious Disease.
3.4.8 Must be ≥ 3 months after prior myeloablative transplant, if applicable

3.5 Exclusion Criteria

3.5.1 Pregnancy or breast feeding
3.5.2 Evidence of HIV infection or known HIV positive serology
3.5.3 Active serious infection
3.5.4 Congenital bone marrow failure syndrome
3.5.5 Previous irradiation that precludes the safe administration of an additional dose of 200 cGy of TBI
3.5.6 CML in refractory blast crisis
3.5.7 Intermediate or high grade NHL, mantle cell NHL, and Hodgkins disease that is progressive on salvage therapy. Stable disease is acceptable to move forward provided it is non-bulky.
3.5.8 Multiple Myeloma progressive on salvage chemotherapy.

4 DONOR ELIGIBILITY

4.1 Donors weighing less than 40 kg (children) will need evaluation by a pediatrician for suitability of the apheresis procedure
4.2 In general good health as determined by the evaluating medical provider
4.3 Appropriate HLA-match to patient

4.4 Able and willing to have up to 3 separate mobilized apheresis collections performed or if unable to undergo apheresis agrees to a bone marrow harvest (requires additional consent)
4.5 Not pregnant
4.6 Agree to undergo donor viral screening panel
4.7 Voluntary written consent

5 TREATMENT PLAN/DONOR PROCEDURES

Refer to section 5.2 for Donor Procedures.

5.1 Patient

5.1.1 Preparative Regimen

All patients will receive allopurinol 300 mg/day starting at day -6 through day 0.

Day	**Treatment**	**ATG*** (see below for patients requiring ATG)
Day –6	Cyclophosphamide 50 mg/kg IV over 2 hours Fludarabine 30 mg/m^2 IV over 1 hour	ATG 15 mg/kg IV every 12 hours for 6 doses (start day -6) Methylprednisolone 1 mg/kg IV prior to each ATG dose
Day –5	Fludarabine 30 mg/m^2 IV over 1 hour	
Day –4	Fludarabine 30 mg/m^2 IV over 1 hour	
Day –3	Fludarabine 30 mg/m^2 IV over 1 hour	
Day –2	Fludarabine 30 mg/m^2 IV over 1 hour	
Day - 1	TBI 200cGy	
Day 0	Transplant	

*The follow subset of patients should receive ATG as part of the preparative regimen:

- Related donor recipients who have not had exposure to combination chemotherapy in the six months preceding transplant
- Unrelated donor recipients who have not had exposure to combination chemotherapy in the three months preceding transplant
- Unrelated donor recipients who have had only a single induction cycle for the treatment of ALL/AML or MDS or CML blast crisis

Recipients with a prior autologous transplant in the year prior to second transplant do not require ATG.

Fludarabine will be given IV over 1 hour day -6 through day -2. Preparation, administration and monitoring will be according to institutional guidelines.

Cyclophosphamide will be administered as a 2 hour intravenous infusion with a high volume fluid flush and mesna per institutional guidelines on day -6.

ATG, for patients meeting one of the above conditions, will be administered IV every 12 hours for 6 doses on days -6, -5, and -4 according to institutional guidelines.

Methylprednisolone 1 mg/kg IV administered immediately prior to each dose of ATG (6 doses).

TBI 200 cGy given in a single fraction will be given on day -1 per institutional guidelines.

Patients will receive prophylaxis for GVHD beginning at day -3 as follows unless another prophylaxis plan is indicated (e.g. co-enrollment with another study).

Cyclosporine A (CSA) will start day -3 and will be administered PO/IV until day +100 maintaining a trough level > 200 ng/mL. The initial dose will be 2.5 mg/kg IV given per institutional practices. 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. If no GVHD, the dose will be tapered 10% per week beginning on day 101.

Mycophenolate mofetil (MMF) 1.5 gram BID or if < 50 kg will be given 15mg/kg PO/IV bid beginning on day -3 (adjust to tablet size). MMF dosing will be monitored and altered as clinically appropriate per University of Minnesota Medical Center - Fairview Pharmacy guidelines. No dose adjustment for liver disease. MMF dosing to be monitored and altered as clinically appropriate by pharm D or physician.

Discontinue MMF at day +30 *or* 7 days after engraftment (3 consecutive days of absolute neutrophil count (ANC) $\geq$ 0.5 x 10^9 /L), whichever day is later. If the patient has acute GVHD requiring systemic therapy, MMF may be stopped 7 days after control of GVHD (e.g. resolution of skin rash, vomiting, and diarrhea).

5.1.2 Hematopoietic Stem Cell Transplantation (day 0)

Peripheral Blood Stem Cells - On day 0, patients will receive an allogeneic transplant using PBSC which are $CD34^+$ selected as the donor graft. The graft will be infused over 15-60 minutes after premedication with acetaminophen 650 mg PO and diphenhydramine 25 mg PO/IV with doses adjusted for pediatric patients.

Related or Unrelated Bone Marrow Cells - A target dose of 3 x 10^8 nucleated cells/kg recipient weight will be collected and infused after arrival and processing. The graft will be infused over 15-60 minutes after premedication with acetaminophen 650 mg PO and diphenhydramine 25 mg PO/IV with doses adjusted for pediatric patients.

The day 0 cell infusion may be shifted to day +1 or day +2 to shift donor collections to weekdays if needed for scheduling purposes. All associated procedures (i.e. G-CSF doses etc.) will shift accordingly.

5.1.3 Supportive Care

All supportive care including antimicrobial prophylaxis directed towards bacteria, fungi and viruses will be per University Of Minnesota Blood and Marrow Program guidelines.

Acute and chronic GVHD will be staged and treated using current University of Minnesota BMT program GVHD protocols

All patients will receive standard supportive transfusion care according to transfusion committee guidelines or as modified based on clinical parameters.

Patients with WBC counts ≤ 2500 any time after stem cell infusion will be started on G-CSF support at Day +5 at a dose of 5 mcg/kg (IV/SQ) daily rounded to vial size until ANC ≥ 2500 for 2 consecutive days.

5.2 Donor Procedures

5.2.1 Peripheral Blood - Mobilized Donor Leukapheresis

G-CSF Administration

Transplant Day	G-SCF
-4	G-CSF 10 mcg/kg SQ
-3	G-CSF 10 mcg/kg SQ
-2	G-CSF 10 mcg/kg SQ
-1	G-CSF 10 mcg/kg SQ
0	G-CSF 10 mcg/kg SQ
+1 (if needed)	G-CSF 10 mcg/kg SQ

Premedication consisting of acetaminophen 650 mg PO (for pediatric donors: 10-15mg/kg/dose PO with a maximum of 650mg) every 8-12 hours should be given beginning the day of G-CSF administration and ending on the last day of apheresis.

Donors may experience any of the following side effects from G-CSF:

Less common

- bone and muscle pain relieved with acetaminophen
- changes in liver function tests

Rare, but can be serious

- injection site reaction (redness, pain, or swelling)
- allergic reaction (fever, chills, skin itching, or feeling flushed) - More serious reactions happen rarely, but can be dangerous. Symptoms can include feeling lightheaded or dizzy (due to low blood pressure), chest tightness, shortness of breath, back pain, or swelling of the face, eyes, tongue, or throat.
- spleen enlargement or rupture – presenting as pain or swelling on the left side under the rib cage or pain in your left shoulder area.
- trouble breathing or coughing up blood

Donors experiencing intolerable symptoms attributed to G-CSF therapy may have a dose reduction according to institutional guidelines.

5.2.2 Apheresis

The collection will be performed by the University of Minnesota Medical Center - Fairview Transfusion Service using standard collections techniques. Peripheral blood mononuclear cells (PBMC) will be collected using a Fenwal CS-3000® Plus blood cell separator (#4R4538) with granulocyte separation chamber and small volume collection chamber (SVCC) (Fenwal Division, Baxter Healthcare, Deerfield, IL).

The cells will be selected for the $CD34^+$ fraction and infused after collection and processing. A target dose of 5 x 10^6 CD 34+ cells/kg, with a minimum of 3 x 10^6 CD34+ cell/kg recipient weight, will be collected by apheresis for transplant. In most cases this dose will be recovered in a single apheresis; however, additional apheresis may be required to achieve the minimum dose.

The apheresis product will be administered to the recipient without cryopreservation on the day(s) of apheresis.

5.2.3 Related or Unrelated Donor Bone Marrow Cells

A target dose of 3 x 10^8 nucleated cells/kg recipient weight will be collected.

6 REQUIRED OBSERVATIONS

Targeted day may be altered if clinically appropriate

6.1 History and Physical Exam of Recipient

A complete medical history with full details of the patient's previous treatment and response including: duration of disease, prior chemotherapy, hormonal therapy or immunotherapy regimens; radiation (including dose and field size); previous complications of therapy including end-organ toxicity and infections; previous CNS disease and therapy; previous plasmapheresis or lymphapheresis.

6.2 History and Physical Exam of Donor

A complete medical history with special reference to infections past and present (especially viral).

6.3 Pre-Transplant Studies

All patients receive routine BMT recipient malignancy work-up, including bone marrow biopsy and evaluation of renal, liver, cardiac and pulmonary function. Patients with a history of MDS or a history of 2 or more consecutive inductions/reinductions to treat acute leukemia or CML blast crisis or prolonged neutropenia of at least 2 months immediately preceding transplant should have a chest CT without contrast to exclude occult fungal infection prior to transplant.

- MDS/CML blast crisis
- 2 or more consecutive inductions
- prolonged neutropenia 2 months prior to BMT

6.4 Post-Transplant Studies

Study tests may be omitted at the physician's discretion

6.4.1 Blood and Bone marrow aspiration and biopsy for chimerism (and malignant markers as appropriate) at days 21, 60, 100, 6 months, 1 year and 2 years post transplantation.

6.4.2 Quantitative immunoglobulins at days 100, 6 months and 1 year post transplantation.

7 EXPECTED TOXICITIES AND COMPLICATIONS -- see Appendix I

8 MANAGEMENT OF SELECTED RISKS

8.1 Donor Lymphocyte Infusion For Deterioration Of Donor Chimerism

Recipients without active GVHD are eligible for DLI for progressive deterioration in donor chimerism not responsive to taper of immune suppression. Refer to the appropriate University Of Minnesota DLI protocol.

For all patients if the decision is made to give DLI, taper CSA over 14 days.

8.2 Donor PBSC Infusion for Graft Failure

Graft failure and pancytopenia requiring consideration of G-CSF stimulated donor PBSC infusion will be defined as:

- WBC < 100 at day 21 or
- ANC < 300 at day 28
- Or secondary neutropenia (ANC < 300 for > 7 days) anytime after day 28

9 ADVERSE EVENT REPORTING

Toxicity and adverse events will be classified according to NCI's Common Toxicity Criteria version 2.0 (CTC v2). A copy of the CTC can be downloaded from http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/ctcv20_4-30-992.pdf

9.1 Definitions

An **adverse event** (AE) is any symptom, sign, illness or experience, regardless of causality, that develops or worsens in severity during the course of the study.

A **serious adverse event** (SAE) is any adverse event, regardless of causality that:

- Results in **death**.
- Is **life-threatening**.
- Requires inpatient **hospitalization or prolongation of existing hospitalization**.
- Results in **persistent or significant disability/incapacity**.

- Is a **congenital anomaly/birth defect**.
- Is an **important medical event**.

Attribution:
- **Unrelated** - The AE *is clearly NOT related* to the intervention
- **Unlikely** - The AE *is doubtfully related* to the intervention
- **Possible** - The AE *may be related* to the intervention
- **Probable** - The AE *is likely related* to the intervention
- **Definite** - The AE *is clearly related* to the intervention

Unanticipated (unexpected) problems/events are those that are *not* already described as potential risks in the consent form, *not* listed in the Investigator's Brochure or *not* part of an underlying disease.

Anticipated (expected) problems are those that are already described as potential risks in the consent form, listed in the Investigator's Brochure or part of an underlying disease. These do NOT meet the IRB's definition of UPIRTSO and should be reported in summary form only at the time of IRB continuing review. For example, if death is an expected outcome, this event should be reported only at the time of continuing review.

Federal regulations [45CFR46.103(b)(5) and 21CFR56.108(b)(1)] require the IRB to ensure that researchers promptly report "any unanticipated problems involving risk to subjects or others" (UPIRTSOs). The University of Minnesota IRB defines a UPIRTSO as <u>any event which in the opinion of the local investigator was unanticipated, reflects new or increased risk to the subjects and was possibly related to the research procedures.</u>

9.2 Adverse Event Monitoring

All patients will be monitored for adverse events during the first 100 days after hematopoietic stem cell transplantation with the reporting of any events meeting the definition of UPIRTSO to the University Of Minnesota IRB per section 9.3. After day 100, monitoring will lessen according to standard transplant follow-up guidelines; however, upon knowledge, the investigator is obligated to report any event that meets the definition of UPRITSO per section 9.3.

In addition, the following events will be reported to the University of Minnesota University Of Minnesota Blood and Bone Marrow Database: graft failure/ autologous recovery, severe acute GVHD (Grades III and IV), relapse, and death.

9.3 Required Adverse Event Reporting to University Of Minnesota IRB and MCC

Agency	Criteria for reporting	Timeframe	Form to Use	Submission address/ fax numbers	Copy AE to:
U of MN IRB	Any event meeting the definition of UPIRTSO	10 Working Days	UMCC SAE	MMC 820	SAE Coordinator mcc-saes@umn.edu
	Any problem meeting the definition of UPIRTSO*	10 Working Days	UPIRTSO		
	All problems/events that do *not* meet the IRB's definition of UPIRTSO* (see next page)	Annually	Summary format		
MCC SAE Coordinator	Any event that counts toward a study stopping rule (refer to section 12.1)	Time of reporting to BMT database	Stopping Rule	SAE Coordinator mcc-saes@umn.edu	Not applicable

The SAE Coordinator will provide the 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).

*** Problems 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

10 STUDY DATA COLLECTION AND MONITORING PLAN

10.1 Data Management

This study will register patients using The Online Enterprise Research Management Environment (OnCore™), a web based Oracle® database. Key study personnel are trained on the use of OnCore and will comply with protocol specific instructions embedded within the OnCore. Patient registration with demographics will be placed in OnCore and other research databases maintained by MCC IT.

In addition, 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.

10.2 Data and Safety Monitoring Plan (DSMP)

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://www.cancer.umn.edu/exfiles/research/dandsmplan.pdf.

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

- The PI will complete and submit twice yearly Trial Progress Report to the Masonic Cancer Center Data and Safety Monitoring Council (DSMC)
- 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 9.3 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).

10.3 Monitoring

The investigator will permit study-related monitoring, audits, and inspections by the Masonic Cancer Center, IRB, government regulatory bodies, and University of Minnesota compliance groups. The investigator will make available all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data, etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.) will be available for trial related monitoring, audits, or regulatory inspections.

10.4 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.

11 ENDPOINTS

11.1 Primary Clinical Endpoint: Engraftment

Peripheral blood and bone marrow chimerism will be studied at day 21, day 60 and day 100. Peripheral blood chimerism will also be evaluated as clinically indicated.

Successful sustained engraftment is defined as primary neutrophil engraftment by day 42 and >90% donor cells at day 100, with or without DLI.

11.2 Safety Endpoints

Development of severe adverse events within 100 days of transplantation
$\geq$ 30% transplant related mortality at 100 days

12 EXPERIMENTAL DESIGN AND STATISTICAL CONSIDERATIONS

The primary objective of this study is to evaluate the efficacy and safety of fludarabine, cyclophosphamide plus TBI as a nonmyeloablative preparative regimen for hematopoietic stem cell transplantation.

As of 10/12/09, 147 patients of a planned 150 were enrolled on this protocol with the current fludarabine dose of 40mg/m^2 Day -6 through -2. We propose to enroll an additional 150 patients on this trial with a new, lower fludarabine dose of 30mg/m^2 Day -6 through -2. Assessment of engraftment, rates of acute GVHD, chronic GVHD, overall survival (OS), treatment related mortality (TRM), and incidence of relapse will be evaluated.

12.1 Stopping Boundaries

There will be monitoring for stopping rules for non-relapse mortality by day 100. Monitoring will be performed separately for related and unrelated donor transplants and for arms 1-6 (standard risk) vs high risk (arm 7). If any of the stopping rules for arms 1-6 are reached, enrollment in all 7 arms will be halted.

Arms 1-6 (Standard Risk Patients – refer to section 3.2):
For the unrelated donors, consideration will be made to close the study if there are 4 of 4, 5 of 6, 6 of 9, 7 of 11, 8 of 13, 9 of 15 or 11 of 20 non-relapse deaths prior to day 100. This boundary has a type I error rate of 0.05 for a rate of 30% and a power of 51% to detect a rate of 50%.

After 1/31/2004, we expect an additional 30 related donor patients to be enrolled. For these patients, consideration will be made to close the study if there are 3 of 4, 4 of 6, 5 of 8, 6 of 11, 7 of 13, 8 of 16, 9 of 18, 10 of 21, 11 of 23, 12 of 26, 13 of 28 or 14 of 30 non-relapse deaths prior to day 100. This boundary has a type I error rate of 0.05 for a rate of 30% and power of 80% to detect a rate of 50%.

As of this renewal (10/12/09), we expect an additional 150 patients to be enrolled. For these patients, consideration will be made to close the study if there are 6 of 6, 7 of 9, 8 of 11, 9 of 14, 10 of 16, 11 of 19, 12 of 21, 13 of 24, 14 of 27, 15 of 29, 16 of 32, 17 of 34, 18 out of 37, 19 out of 39, 20 out of 42, 21 out of 44, 22 out of 47, 23 out of 49, 24 out of 52, 25 out of 54, 26 out of 57, 27 out of 59, 28 out of 62, 29 out of 64, 30 out of 67, 31 out of 69, 32 out of 72, 33 out of 74, 34 out of 77, 35 out of 79, 36 out of 84, 38 out of 87, 39 out of 89, 40 out of 92, 41 out of 95, 42 out of 97, 43 out of 100, 44 out of 102, 45 out of 105, 46 out of 107, 47 out of 110, 48 out of 112, 49 out of 115, 50 out of 117, 51 out of 120, 52 out of 122, 53 out of 125, 54 out of 127, 55 out of 130, 56 out of 132, 57 out of 135, 58 out of 137, 59 out of 140, 60 out of 142, 61 out of 145, 62 out of 147 and 63 out of 150 non-relapse deaths prior to day 100. This boundary has a type I error rate of 0.05 for a rate of 30% and power of 80% to detect a rate of 50%.

For Arm 7 (High-Risk Patients – refer to section 3.3):
Continuous stopping boundaries have been designed to stop enrollment if the Day 100 non-relapse mortality is significantly higher than 30%. The trial will be stopped if 4 of 4, 5 of 6, 6 of 9, 7 of 11, 8 of 13, 9 of 15, or 11 of 20 patients experience non-relapse mortality by Day 100. This boundary has a type I error of 0.051 for a D100 mortality rate of 30%, and a power of 51% to detect a rate of 50%.

12.2 Statistical Analysis

The primary outcome variable is (P), the proportion of patients with engraftment. Patients who do not have sustained engraftment will be considered to have graft failure.

Patients who die before day 21 will be considered non-evaluable for engraftment and the cause of death noted.

Secondary outcomes include survival, non-relapse mortality, and grade III-IV acute GvHD. Survival will be estimated by Kaplan-Meier methods. Non-relapse mortality and acute GvHD will be estimated using cumulative incidence, treating non-event deaths as a competing risk.

12.4 Rationale for Sample Size

We estimate that we can enroll about 25 patients per year.

13 ETHICAL CONSIDERATIONS

13.1 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.

13.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. The IRB will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB approval has been obtained. The protocol, informed consent, written information given to the patients, safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB by the investigator.

13.3 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, he/she 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.

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APPENDIX I: TOXICITIES AND COMPLICATIONS

Toxicities Associated with the Preparative Regimen:

Cyclophosphamide –

Common	Less Common	Rare
• low white blood cell count • hair loss or thinning, including face and body hair • nausea • vomiting • loss of appetite • sores in mouth or on lips • bleeding from bladder, with blood in urine • diarrhea • long-term or short-term infertility in women and men	• low platelet count • darkening of nail beds • acne • tiredness • infection • fetal changes if pregnancy occurs while taking cyclophosphamide	• heart problems with high doses, with chest pain, shortness of breath, or swollen feet • severe allergic reactions • skin rash • scarring of bladder • renal tubular necrosis which can lead to kidney failure • scarring of lung tissue, with cough and shortness of breath • second cancer • death from infection, bleeding, heart failure, allergic reaction, or other causes

Fludarabine -

Common	Less Common	Rare
• severe suppression of blood counts • fatigue • nausea • vomiting • fever and chills • infection	• pneumonia • diarrhea • loss of appetite • weakness • pain	• numbness and tingling in hands and/or feet related to irritation of nerves • changes in vision • agitation • confusion • clumsiness • seizures • coma • cough • trouble breathing • intestinal bleeding • weakness • death due to effects on the brain, infection, bleeding, severe anemia, skin blistering, or other causes

Total Body Irradiation (TBI) -

Common	Less Frequent	Uncommon
• diarrhea • nausea • stomach cramps • vomiting • painful swelling of the parotid gland for a few days • short-term hair loss • anemia • infection • bleeding • cataracts • sterility • growth failure • endocrinopathies (such as thyroid disease or diabetes) • mouth sores	• pneumonia • redness of the skin • lowered liver function	• risk of developing other cancers in the future • difficulty swallowing • back problems (deformities in the vertebrae) • lowered kidney function

Thymoglobulin (ATG)

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

Toxicities associated with GVHD Prophylaxis:

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

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)

APPENDIX II: CHEMOTHERAPY DRUG INFORMATION

Cyclophosphamide (Cytoxan)
Formulation: parenteral 100 mg and 500 mg vials. Commercially available
Availability: Commercially available
Storage: room temperature
Mixing: IV drug should be mixed with sterile water for IV use. Dissolves very slowly with cold water.
Administration: Cytoxan is added to 250 ml of D5W and administered IV drip over a 1-2 hour period usually at 10 am. Use an appropriate smaller volume for children.
Toxicity: Nausea and vomiting, alopecia, bone marrow depression, immunosuppression, hemorrhagic cystitis, azospermia.

Fludarabine (Fludara- Berlex) (F-Ara-A, fludarabine phosphate)
Formulation: Vials (lyophilized solid cake): 50 mg (25 mg/ml after reconstitution)
Availability: Commercially available
Storage: Between 2 and 8° centigrade (36 and 46 F)
Mixing: Adding 2 mL of sterile water for injection to the 50 mg vial, producing a solution containing 25 mg/mL (the solid cake should fully dissolve within 15 seconds). Solutions may be further diluted in 100 or 125ml of 5% dextrose injection or 0.9% sodium chloride injections for administration by intravenous infusion.
Stability: Once diluted, solutions should be promptly administered or stored in the refrigerator for no more than 8 hours prior to administration.
Administration: Fludarabine is administered over a 1-hour period.
Toxicity: Nausea and vomiting, bone marrow depression, fever, immunosuppression.

APPENDIX III: RESEARCH RELATED CONTROL COHORT FOR ITREG STUDY (MT2012-06)

In November 2013, MT2012-06: "Dose Escalation Study with Extension of Inducible Regulatory T Cells (iTregs) in Adult Patients Undergoing Non-Myeloablative HLA-Identical Sibling Donor Peripheral Blood Stem Cell Transplantation" (M MacMillan, PI) opened to enrollment. As MT2012-06 recruits a sub-set of the same patient population as does MT2001-10, this sub-study serves to enroll 10 MT2001-10 patients who consent to act as controls for the "iTreg patients" by providing additional blood for research related testing prior to the start of the preparative regimen, prior to the transplant on day 0, and at 7 time points over the 1st 100 days post-transplant.

This is an optional sub-study for persons 18 years and older enrolling on MT2001-10 with one of the following diagnoses for which a related donor will be used as the stem cell source:

- Acute myelogenous leukemia
- Acute lymphocytic leukemia
- Chronic myelogenous leukemia
- Non-Hodgkin lymphoma or Hodgkin lymphoma
- Myelodysplastic syndrome

The samples will be used to assess for the number of T regs and T effector cells at each time point compared with the patients enrolled on MT2012-06. The need for the control is to be able to determine what contribution the iTregs have versus the PBSC. As the Tregs are from the same donor as the peripheral blood stem cell (PBSC) graft the comparison is not possible without a control.

Research Related Sample Collection Schedule

Activity/ Laboratory		Pre Chemo	Days Post-HCT (+/- day)					
		-6 or earlier	+0 Pre HCT	+1 (±1)	+3 (±1)	+7 (±1)	+14 (±1)	+28 (±3), +60 (±7), +100 (±7)
Consent for additional sample collection	Pre-HCT							
5 – 10 ml green top tubes 1 – 5 ml red top tube To TTL		X	X	X	X	X	X	X