THE UNIVERSITY OF CHICAGO

DIVISION OF MEDICINE

Combined Haploidentical-Cord Blood Transplantation for Adults and Children

APPENDIX I: ALLO DONOR SELECTION CRITERIA
APPENDIX J: APHERESIS EVALUATION AND MANAGEMENT

STUDY CHAIRMEN:

Andrew Artz, M.D.	Amittha Wickrema, Ph.D.

CO-INVESTIGATORS:

Wendy Stock, M.D.	Hongtao Liu, M.D., Ph.D
Richard Larson, M.D.	Toyosi Odenike, M.D.
John Cunningham, M.D.	Lucy Godley, M.D., Ph.D.
Justin Kline, M.D.	

STATISTICIAN: Theodore Karrison, Ph.D.

RESEARCH NURSE: Paula Del Cerro, R.N., O.C.N.

DATA MANAGEMENT: Scott Allen, CRA

Patricia Popa, CRA

REGULATORY AFFAIRS MANAGER: Inga Krueger, MA, CCRP

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1.0 OBJECTIVES:

1.1 Primary Objective

To assess the rate of engraftment with combined haploidentical-cord blood transplantation.

1.2 Secondary Objective

To evaluate the incidence and severity of acute and chronic GVHD.

2.0 BACKGROUND

2.1 Umbilical cord blood transplantation in hematologic malignancies

Transplantation of hematopoietic stem cells derived from bone marrow and peripheral blood of sibling and unrelated donors has been used successfully to treat patients with high-risk or recurrent hematological malignancies. However, bone marrow transplants (BMT) are limited by the lack of human leukocyte antigen (HLA) matched donors and the high risk of graft-versus-host disease (GVHD) after transplantation. Although there are more than 10 million registered donors worldwide, more than 30% of patients requiring transplants are unable to find a matched donor.[1] This proportion is higher for patients of minority descent. Patients who receive unmatched transplants have decreased survival secondary to severe GVHD (80%) and opportunistic infections.[2]

In order to increase the donor pool, umbilical cord blood (UCB) is now being used for transplants. Unrelated UCB transplants have a high rate of engraftment with a low risk of acute GVHD even in the 1-2 HLA mis-matched setting in children[3-5] and adults.[6] However, delayed engraftment and associated treatment-related toxicity and opportunistic infections result in high treatment-related mortality in adults. Laughlin et al emphasized the importance of nucleated cell dose in hematopoietic recovery while HLA compatibility played a less significant role. Clearly demonstrated was an increased event-free survival rate in patients who received grafts containing more than 1.2×10^5 CD34+ cells per kilogram.[6] Recently the Institute of Medicine commissioned a study of UCB outcomes from pooled data of 755 cases of adults and children in the United States. This study showed a clear effect of HLA matching on survival, with 6/6 matches faring better than 5/6 or 4/6 matches. The magnitude of this effect, however, was most apparent at lower cell doses ($<2.5 \times 10^7$ total nucleated cells per kilogram), emphasizing the effect of cell dose relative to HLA match.[7]

2.2 Double cord blood transplantation, Haploidentical transplantation, and Combined Haploidentical and Cord Blood transplantation

Disappointed with the delayed engraftment after cord blood transplantation, several groups have tried to enhance recovery by (1) *in vitro* expansion of cords;[8] (2) infusion of multiple cords;[9] or (3) combination of cord blood transplant with cells from other

origins. One way to increase the nucleated cell dose is to combine UCB units. Initial animal studies have shown that combined grafts have a higher survival than single grafts.[10] In a pilot series of patients with hematologic malignancies, Barker et al showed that combined transplantation of two partially matched UCB units resulted in a median time to neutrophil engraftment of 23 days (range 15-41).[9] Interestingly, in each case, permanent hematopoiesis was derived from one of the two cords. The other cord was eliminated. While total nucleated nor CD34+ cell counts nor HLA match predicted which donor would predominate, the predominating unit had a significantly higher CD3+ dose ($p<0.01$). Nucleated cell and CD34+ dose in the predominating unit were significantly associated with speed of neutrophil recovery. With a median follow-up of 10 months (range 3.5 months-2.5 years), disease-free survival (DFS) was 57% at one year, with 72% of patients alive at one year if transplanted in remission or chronic phase.[11] Ballen et al reported results of a phase 1 study of double cord transplantation using a reduced intensity conditioning regimen of fludarabine, melphalan, and rabbit antithymocyte globulin (rATG, Thymoglobulin).[10] The median time to neutrophil engraftment was 20 days (range 15-34). The 100 day transplant-related mortality was 14%. Chimerism analysis showed predominance of one cord by Day +100 in 79% evaluable patients. In 85% of these patients, the first cord infused predominated. With a median follow-up of 7 months (range 2-16 months), the overall survival was 79%, and the DFS was 64%. Two patients (both with MDS complicating aplastic anemia) experienced primary graft failure.[11]

Haploidentical transplantation, the transplantation of stem cells from a partially matched related donor (typically a parent, child or sibling) has been explored starting in the early 1980's. Initial studies were characterized by a high incidence of graft failure, immunodeficiency, and GVHD.[2] The administration of massive doses of CD34-selected (thus T-cell depleted) stem cells can prevent graft rejection, perhaps through a veto mechanism, and assure reliable engraftment without excessive GVHD.[12] In clinical trials of pediatric and young adult patients, this has resulted in acceptable outcomes.[13] We have previously tested this approach in adults with advanced hematologic malignancies with disappointing results. Reliable and rapid engraftment occurred, and the incidence of GVHD was very low. But patients had prolonged severe immunosuppression, and there was a high incidence of delayed fatal opportunistic infections.[14]

In order to augment recovery of hematopoiesis following cord blood transplantation, a Spanish group has studied combined haploidentical and cord blood transplantation. Their observations indicated that initial rapid engraftment was obtained from the haploidentical T-cell depleted graft; subsequent hematopoiesis resulted from the cord blood which appeared to permanently replace the haploidentical hematopoiesis.[15]

In an oral presentation at the 2004 American Society of Hematology Meetings[16], Fernandez summarized results of this work, which has been updated at the 2005 Third Annual International Umbilical Cord Blood Transplantation Symposium[17] and in a recent publication[18]. His group has transplanted 28 patients with advanced hematologic malignancies. The median age was 30 (16-63) and weight 67 (43-87) Kg. The basic

conditioning regimen was fludarabine, cyclophosphamide, total body irradiation (TBI), and equine antithymocyte globulin (ATGAM), with modifications in 6 patients. The UCB units had 2.37 (1.31-3.7) $x10^7$ TNC/Kg and 0.11 (0.035-0.37) $x10^6$ CD34+ cells/Kg and were 0-2 HLA mismatches to the recipient. The 3rd party donor was an HLA haploidentical relative for 24 (mother for 4; also father, sibling, son, nephew), a higher HLA mismatched (mm) relative for 3, and a fully HLA mm unrelated donor for 1. All 3rd party cells were extensively T-cell depleted so that the infused cells contained a median of 2.31 (1.05-2.58) $x10^6$ CD34+/Kg and 0.25 (0.05-0.98) $x10^4$ CD3+/Kg.

Initially, post-transplant circulating granulocytes were predominantly from the 3rd party donor in all patients except the four receiving maternal CD34+ cells. Analysis of DNA polymorphisms showed initial predominance of the 3rd party donor both in granulocytes and mononuclear cells and subsequent progressive replacement by UCB cells. Excluding the 4 patients who received maternal mobilized peripheral blood cells, the corresponding data was: median time to ANC>$0.5x10^9$/L was 9.5 days (9-17); CB-ANC>$0.5x10^9$/L was 22 days; and full CB chimerism 54 days; median time to platelets >$20x10^9$/L and >$50x10^9$/L was 30 and 54 days, respectively. The 4 who received maternal cells had no significant engraftment of these cells, although 3 had exclusive UCB chimerism, 2 of which reached full UCB engraftment (days 20 and 36). Replacement of the 3rd party graft by the UCB engraftment may be due to rejection by the UCB-derived immune system or immunization to minor histocompatibility antigens.[18]

20 of 27 (74%) patients had acute GVHD (a-GVHD) of any grade after day 11 post-transplant; 4 of 27 (14.8%) reached grade III-IV. Most of the patients with a-GVHD responded to corticosteroid therapy. There were two deaths primarily related to a-GVHD, one occurred in one of two patients who did not receive ATG as part of the preparative regimen. DNA analysis of skin biopsies of GVHD lesions showed UCB DNA in several instances, but in no case were third party donor cells detected. Only 4 out of 20 patients at risk developed manifestions of chronic GVHD (c-GVHD), which was limited in all.

8 deaths have been reported related to the procedure (toxicity 2; graft failure 1; GVHD >II 2; opportunistic infection 3). The 2 deaths due to toxicity occurred in patients who received 1.2 Gy TBI without fludarabine. One developed multi-organ failure and the other veno-occlusive disease. Two of the deaths due to opportunistic infection were due to CMV; the other from toxoplasmosis. With a median follow up of 10 months (1-75) for all patients and 16 months (3-75) for the 20 living (9 surviving >2years), the 4 year overall survival is 67% for the whole group.[17]

2.3 Rationale for the current study

The applicability of BMT is limited by the availability of a matched donor. As described earlier, umbilical cord transplants provide an additional source for hematopoietic stem cells (HSCs). Partially matched UCB transplants have already been shown to engraft with reduced acute GVHD. The major limitation to UCB

transplants is delayed engraftment which is related to nucleated cell dose. Our patient population is relatively older, has advanced disease, and often suffers from comorbidities. Prolonged neutropenia is not tolerated, and intensive conditioning is necessary to induce durable disease control. We are therefore interested in recapitulating the Spanish data in a patient population with more advanced disease.

For patients transplanted in remission, we will use a conditioning regimen of fludarabine-melphalan because it is relatively well tolerated and effective in assuring engraftment and preventing GVHD. [11, 19]

For high risk patients, we will use either fludarabine-thiotepa-TBI[20] or busulfan-fludarabine[21], as currently evaluated at University of Chicago.

Correlative studies will include those related to engraftment and immune reconstitution.

The essential differences between our proposed study and the Spanish study will be the following:

1. Patient selection. The median age in our studies is usually over 50, more than two decades older than in the Spanish studies. Age-related differences in the immune system may profoundly affect our outcomes.

2. Differences in conditioning regimen, which are influenced by our institutional experience.

2.4 Preliminary Data as of February 2012

We published our initial experience with reduced intensity conditioning of this regimen (Liu, Blood, Oct 2011 pre-published online). Overall, this regimen has been promising with early engraftment in the majority of subjects and cord-blood predominance later. We also had shifted from TBI based regimens for active disease to an alternative non-radiation approach of disease reduction before transplant with chemotherapy and then at the nadir of cytopenia, using reduced intensity conditioning. This is an approach we have used for matched donors with success.

3.0 PATIENT ELIGIBILITY

3.1 Inclusion criteria

Patients will be eligible for this study if they have any one of the diseases that are known to be cured after allogeneic stem cell transplantation. For example:

1. Relapsed or refractory acute leukemia (myeloid or lymphoid)
2. Acute leukemia in first remission at high-risk for recurrence
3. Chronic myelogenous leukemia in accelerated phase or blast-crisis
4. Chronic myelogenous leukemia in chronic phase intolerant, refusing, or refractory to TKI.
5. Recurrent or refractory malignant lymphoma or Hodgkin lymphoma
6. Chronic lymphocytic leukemia, refractory, relapsed or with poor prognostic features (based on karyotype or Ig Mutation profile)
7. Multiple myeloma failing or not eligible for autologous transplant
8. Myelodysplastic syndrome with high risk feature (based on accepted risk features such as IPSS or failure of other treatment)
9. Chronic myeloproliferative disease with high risk features and/or failures of conventional treatment.
10. Hemoglobinopathies with high risk features (frequent vaso-occlusive crisis, stroke etc.).
11. Aplastic anemia failing conventional treatment such as immunosuppressants or not appropriate for conventional treatments.

3.2 Exclusion criteria

Patients must be discussed and approved at the weekly Stem Cell Transplant conference after careful consideration of the following criteria:

1. Zubrod performance status > 2 (see Appendix E)
2. Life expectancy is severely limited by concomitant illness
3. Patients with severely decreased LVEF or impaired pulmonary function tests (PFT's)
4. Estimated Creatinine Clearance <50 ml/min
5. Serum bilirubin> 2.0 mg/dl or SGPT >3 x upper limit of normal
6. Evidence of chronic active hepatitis or cirrhosis
7. Active HIV-infection defined as positive viral load in past 6 months, not being on a stable anti-retroviral regimen for 6 months, and/or opportunistic infections (excluding treatment for responsive thrust, mycobacterium and herpes infections)
8. Patient is pregnant
9. Patient or guardian not able to sign informed consent

4.0 PATIENT SELECTION AND STEM CELL SOURCE

4.1 Patient selection

Patients are eligible for enrollment if they are an appropriate candidate for transplantation, and an HLA-identical related or unrelated donor cannot be identified within an appropriate time frame.

4.2 Hematopoietic stem cell source

Umbilical cord cells will be obtained from established umbilical cord blood centers. Umbilical cord preparation procedure will be according to established cell lab procedures in accordance with FACT/FDA guidelines.

4.3 Selection of grafts

4.3.1 Cord Blood. Preliminary searches of umbilical cord-blood banks will be performed using the patient's HLA phenotype, as determined by molecular typing for class I HLA-A, B, C; and class II DR and DQ. Preferred cord-blood units are those that are antigen-matched for greater than 3 of 6 HLA loci (HLA A, B, DR) and contain a minimum cell count of 1×10^7 nucleated cells per kilogram of the recipient's body weight before freezing. A less closely matched graft with higher nucleated cells will be selected over a more closely matched graft with fewer nucleated cells.

4.3.2 Third Party Donor. The preferred 3rd party donor will be a young, non- maternal HLA haploidentical relative. After appropriate evaluation as per transplant program critera (Appendix I) , the donor will receive G-CSF (filgrastim) 5 mcg/kg SQ BID for four consecutive days. Apheresis will start on the morning of the fifth day and proceed until sufficient cells have been collected. Apheresis procedure will be conducted as per transplant program policy (Appendix J) Typically four total blood volumes (TBV) are collected. The use of pediatric donors is restricted to donors who are over the age of 14 and weigh more than 50 kg.

5.0 TREATMENT PLAN

5.1 All patients shall be registered with the Data Management Office

Register patients in theVelos system (http://velos.bsd.uchicago.edu/eres/jsp/ereslogin.jsp)

5.2 Conditioning Regimens.

5.2.1 Good Risk Patients (refer to ASBMT Criteria[22])**:**

- **Fludarabine-Melphalan** as per institutional guidelines (See Appendix A)
- **Rabbit antithymocyte globulin** (Thymoglobulin, r-ATG)

5.2.2 High Risk Patients (refer to ASBMT Criteria[22])**:**

5.2.2.1 Fludarabine-Thiotepa-TBI as per institutional guidelines (See Appendix B).

Rabbit antithymocyte globulin (Thymoglobulin, r-ATG)

5.2.2.2 Busulfan-Fludarabine as per institutional guidelines will be considered if the patient is not eligible for TBI (See Appendix C). Busulfan will be targeted if possible to an AUC of 4800 to 6000 per day using therapeutic drug monitoring

Rabbit antithymocyte globulin (Thymoglobulin, r-ATG)

5.2.2.3 Cytoreductive therapy at the treating physicians discretion followed 7 – 21 days later (although longer if clinically required)

- **Fludarabine-Melphalan** as per institutional guidelines (See Appendix A)
- **Rabbit antithymocyte globulin** (Thymoglobulin, r-ATG)

5.3 Umbilical cord blood infusion

On day 0 or day 1 the cord blood product will be prepared and infused according to transplant program policy 431.02 "Infusion of Cryopreserved Hematopoietic Progenitor Cells by Nurses" (see Appendix H).

5.4 Haploidentical stem cell preparation

- Stem cells will be collected from a haploidentical donor. The preferred 3rd party donor will be a young HLA haploidentical relative. After appropriate evaluation as per transplant program criteria, the donor will receive G-CSF (filgrastim) 5 mcg/kg SQ BID daily for four consecutive days. (doses rounded to the nearest vial size) Apheresis will start on the morning of the fifth day and proceed until sufficient cells have been collected.
  Based on Spanish experience, the maternal donor will be avoided.

- After collection and prior to cryopreservation, cells will be T-cell depleted using the **Isolex** 300i CD34 depletion device. The target will be to obtain a product containing less than $1x10^4$ CD3+ cells per kg of recipient body weight and no more than $3x10^6$/kg CD34 positive cells. The haploidentical cells will be infused on day 0 or day 1 according to transplant unit policy.

- As of early April 2010, the Isolex 300 CD34 depletion device is no longer commercially available and instead we use the Miltenyi Clinimax device which serves a similar purpose. An IND has been obtained that covers both the device and the need for all cord bloods to be licensed or fall under an FDA IND.

5.5 GVHD prophylaxis

- Tacrolimus 0.03 mg/kg/day IV CI over 24 hr from 4 PM day -2 until engraftment or when patient is able to take PO, then tacrolimus approximately 0.09 mg/kg PO in 2 divided doses. Tacrolimus should be given at full dose to maintain levels of 5-15 ng/mL through day **180**. Thereafter, tacrolimus will be tapered by 20% every week.

- Additional GVHD prophylaxis according to standard of care.

5.6 Supportive care

- Infection prophylaxis and supportive care will be as per BMT unit policy.

- All patients, regardless of disease histology will receive G-CSF 5 mcg/kg (rounded to 300 mcg or 480 mcg, depending on patient weight) SQ daily from day 1 until ANC >5 x10E9/L.

- Suggested premedication for rATG will consist of diphenhydramine, acetaminophen, and methylprednisolone.

6.0 PRETREATMENT EVALUATION (See TABLE I)

6.1 EVALUTION OF THE PATIENT (The following list is to be used as a guideline, but can be adjusted in individual cases. Please also refer to transplant policies.)

1. Complete history and physical examination; height/weight; performance status
2. Bone marrow biopsy and aspirate with leukemia markers, cytogenetics, flow cytometry
3. CBC, differential, platelets
4. Complete metabolic profile (including complete liver function panel); magnesium, phosphate, LDH, uric acid
5. PT and PTT
6. Hepatitis screen
7. Serum titers for CMV, HSV, EBV
8. HIV antibody
9. Complete urinalysis

10. Urine pregnancy test (if female)
11. Baseline EKG
12. MUGA with measurement of LVEF
13. Pulmonary function tests with DLCO
14. Chest X-ray
15. CT scan of the sinuses (without IV contrast)
16. CT scan of chest/abdomen/pelvis (routinely without IV contrast; with IV contrast at the discretion of the treating physician)
17. HLA class I and class II molecular typing
18. ABO and Rh typing
19. Peripheral blood for chimerism studies
20. Lumbar puncture for cell count, protein, glucose, and cytology (in patients with ALL and high grade NHL, or if prior history of CNS involvement)
21. Quantitative immunoglobulins, serum free light chains, serum and 24-hour urine protein electrophoresis and immunoelectrophoresis, skeletal survey (for multiple myeloma patients only
22. Any additional tests that may be required for clinical care and described in the transplant policies.
23. 20 cc of blood and 5 cc of bone marrow will be stored on all recipients to be used for research purposes

6.2 EVALUATION OF THE DONOR (The following list is to be used as a guideline, but can be adjusted in individual cases. Please also refer to transplant policies.)

1. Complete history and physical examination; performance status
2. CBC, differential, platelets
3. Complete metabolic panel (including complete liver function tests), magnesium, phosphate
4. PT and PTT
5. Hepatitis screen
6. Serum titers for CMV, HSV, EBV; CMV PCR
7. HIV antibody
8. Complete urinalysis
9. EKG
10. Chest X-ray
11. HLA Class I and Class II molecular typing
12. ABO and Rh typing
13. Peripheral blood for chimerism studies

7.0 <u>EVALUATION DURING STUDY</u> (See TABLE I) (The following list is to be used as a guideline, but can be adjusted in individual cases. Please also refer to transplant policies.)

7.1 EVALUATION DURING THE FIRST 100 DAYS

- Evaluation during the first 100 days will be done as per transplant program guidelines.
- Restaging of disease and engraftment studies will be performed between day 25 and day 35.

7.2 DAY 100 EVALUATION (The following list is to be used as a guideline, but can be adjusted in individual cases. Please also refer to transplant policies.)

1. Review of systems and physical examination; performance status
2. Bone marrow biopsy and aspirate with leukemia markers, cytogenetics, flow cytometry
3. CBC, differential, platelets
4. Complete metabolic profile (including complete liver function panel); magnesium, phosphate, LDH
5. CMV, BK, EBV, adenovirus PCR
6. Chest X-ray
7. Pulmonary function tests with DLCO
8. Schirmer's test and slit lamp examination
9. Peripheral blood for chimerism studies
10. Quantitative immunoglobulins, serum and 24-hour urine protein electrophoresis and immunoelectrophoresis, skeletal survey (for multiple myeloma patients only
11. Restaging of disease as indicated by disease specific testing

7.3 EVALUATION DAYS 100-365 (patient without chronic GVHD)

Physical examination and screening labs at least monthly through day 365
Restaging of disease at Day 180 and one year after transplant; or at relapse
Follow-up for patients with chronic GVHD as per chronic GVHD guidelines

7.4 ANNUAL EVALUATION (The following list is to be used as a guideline, but can be adjusted in individual cases. Please also refer to transplant policies.)

1. Review of systems and physical examination; performance status
2. Bone marrow biopsy and aspirate with leukemia markers, cytogenetics, flow cytometry
3. CBC, differential, platelets
4. Complete metabolic profile (including complete liver function panel); magnesium, phosphate, LDH
5. Chest X-ray
6. Pulmonary function tests with DLCO
7. Thyroid function tests

8. Schirmer's test and slit lamp examination
9. Peripheral blood for chimerism studies
10. Quantitative immunoglobulins, serum and 24-hour urine protein electrophoresis and immunoelectrophoresis, skeletal survey (for multiple myeloma patients only
11. Restaging of disease as indicated by disease specific testing

7.5 RESEARCH BLOOD AND BONE MARROW SAMPLES (SEE APPENDIX G)

7.5.1 Samples of blood and bone marrow will be obtained at the following time points:

Prior to admission
Day 0 (Blood)
Day 7 (Blood)
Day 10(Blood)
Day 14 (Blood)
Day 28 (BM and Blood)
Day 100 (BM and Blood)
Day 180 (BM and Blood)
At relapse (BM and Blood)
One year and yearly thereafter (BM and Blood)

7.5.2 Chimerism will be determined by molecular analysis of peripheral blood and bone marrow samples. Peripheral blood chimerism studies will be performed every two weeks for the first 100 days following haploidentical-cord blood transplantation. Samples of blood and bone marrow will be obtained at the following time points:

Day 14 (Blood)
Day 28 (BM and Blood)
Day 42 (Blood)
Day 56 (Blood)
Day 70 (Blood)
Day 84 (Blood)
Day 100 (BM and Blood)
Day 180 (BM and Blood)
At relapse (BM and Blood)
One year and yearly thereafter (BM and Blood)

7.5.3 Immune reconstitution will be assessed by lymphocyte subset analysis of blood per transplant program guidelines.

7.5.4 Bone marrow samples -2 green top tubes will be stored at the time of diagnostic bone marrow aspirations. Request this sample on the bone marrow requisition in the "Other " section.

7.5.5 A back-up stem cell collection will be considered for patients who are in remission.

Table I
SCHEDULE OF PATIENT TESTS

Tests & Observations	**Prior to Study**	**Day of Transplant**	**Day 30 (± 1 wk) Post Transplant**	**Day 100 (± 1 wk)**	**Day 180 (± 1 wk)**	**Post-Tx Follow-up****
History and Progress Notes	X	X	X	X	X	X
Physical Examination	X	X	X	X	X	X
Pulse, Blood Pressure	X	X	X	X	X	X
Height/Weight/BSA	X					
Performance Status	X	X	X	X	X	X
Tumor Measurements	L	L	L	L	L	L
Toxicity Assessment		X	X	X	X	X
AGVHD Assessment			X	X	X	
CGVHD Assessment					X	X
Laboratory Studies						
CBC, Differential, Platelets	X	X	X	X	X	X
Serum electrolytes	X	X	X	X	X	X
Serum creatinine, BUN	X	X	X	X	X	X
AST, ALT, Bilirubin	X		X	X	X	X
LDH	X		X	X	X	X
PT/PTT	X					
Hepatitis Screen	X					
Serologies: CMV, HSV, EBV	X					
CMV PCR	X		X	X		
HIV Ab	X					
Urinalysis	X					
Serum or urine HCG, if female	X					
Quantitative immunoglobulins	M		M	M		M
SPEP, UPEP, SIEF, UIEF	M		M	M		M
HLA class I and II molecular typing	X					
ABO and Rh Typing	X					

Tests & Observations	Prior to Study	Day of Transplant	Day 30 (± 1 wk) Post Transplant	Day 100 (± 1 wk)	Day 180 (± 1 wk)	Post-Tx Follow-up**
Surveillance Tests						
ECG	X					X
MUGA with LVEF	X					X
PFTs with DLCO	X			X		X
Thyroid function tests						X
Schirmer test and slit lamp exam				X		X
LP with cell count, gluc. prot, cytology	H					
Screening						
Chest x-ray, PA & Lateral	X			X		X
CT sinus	X					
CT chest/abd/pelvis	X		L	L	L	L
Skeletal survey	M			M		M
Bone Marrow Asp & Biopsy	X		X	X		X
Cytogenetics	X		X	X		X
Flow cytometry	X		X	X		X
Chimerism						
Peripheral blood (20 cc min.)	X		X	X	X	X
Bone marrow aspirate			X	X	X	X
Immune Reconstitution						
Peripheral blood (40 cc min.)*	X	X	X	X	X	X

* Additional samples on day 7, day 14, day 50, day 75 and then 3 samples/year for a maximum of 5 years.

** At one-year post-transplant, and then yearly thereafter for a maximum of 5 years from study entry, and at relapse.

H: ALL, high grade NHL, or past history of CNS involvement

L: For lymphoma patients.

M: For multiple myeloma patients.

8.0 CRITERIA FOR STUDY EVALUATION

- **Event Free Survival**

  Relapse will be recorded by the day of initial detection of malignant cells if these cells were on subsequent testing confirmed to be increasing in number. The molecular detection of MRD will not be taken into account for the definition of clinical recurrence. The diagnosis of disease recurrence will be based on clinical and pathological criteria.

- **Toxicity**

  Toxicity will be scored according to NCI-CTC criteria.

  Acute GVHD will be scored according to the criteria proposed by Przepiorka et al. (Appendix F).[23]

  Chronic GVHD will be scored according to the NIH Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: I. Diagnosis and Staging Working Report (Appendix G and Appendix H).[24]

- **Definitions of Engraftment**

  Myeloid engraftment will be defined as the first day in which the ANC is > 500/mm^3 for three consecutive days. Cytogenetic and chimerism studies will be performed to confirm donor origin.

  Platelet engraftment will be defined as the first day the platelet count is > 20,000/mm^3 without transfusion support for seven consecutive days.

  Failure to engraft will be defined as lack of evidence of hematopoietic recovery (ANC < 500/mm^3 and platelet count < 20,000/mm^3) by day +35, confirmed by a biopsy revealing a marrow cellularity < 5%. Graft failure will also be defined as initial myeloid engraftment by day +35, documented to be of donor origin, followed by a drop in the ANC to < 500/mm^3 for more than three days, independent of any myelosuppressive drugs, severe GVHD, CMV, or other infection.

  There may be rare cases in which the haploidentical transplant is rejected, but the cord transplant engrafts with delay. Loss of established haploidentical engraftment will be considered graft failure.

  Graft rejection will be defined as graft failure with documentation of return of recipient hematopoiesis as determined by cytogenetic and/or chimerism studies.

  Chimerism studies will be used to determine the percentage cord vs haploidentical donor engraftment.

9.0 STATISTICAL CONSIDERATIONS

The primary endpoint of this trial is the rate of engraftment (which is the opposite of graft failure). Up to 53 patients will be enrolled in the trial. Graft failure will be monitored using the group sequential procedure described in O'Brien and Fleming[25] to test the null hypothesis that the graft failure rate is $\leq$ 10%, against the alternative that the rate is > 10%. Four interim analyses will be performed after n=10, 20, 30, and 40 patients have been enrolled, followed by a final analysis at n=53 using a simple z-test. For a one-sided test at the overall 0.10 alpha level, the critical z-values are 3.50, 2.24, 1.87, 1.59, and 1.41, respectively. Applying a continuity correction, this implies that if 5 or more of the first 10 patients, 6 or more of the first 20, 7 or more of the first 30, or 8 or more of the first 40 patients experience graft failure, the trial may be terminated. The null hypothesis will also be rejected if, at the end of the trial, 9 or more graft failures have occurred. Rejection of the null hypothesis will indicate that the graft failure rate is unacceptably high, i.e., the rate of engraftment is too low. The test has 80% power if the true graft failure rate is 20%.

GVHD is the secondary endpoint, and we are particularly interested in monitoring high risk extensive GVHD. We will review the data after the first 5, 10, 15, and 20 patients with successful engraftment and without evidence of relapse reach their 18-month landmark. Evidence that the rate of high-risk, extensive GVHD in this subset of patients is >40% will lead to consideration for early stopping. Specifically, if 4 of 5, 6 of 10, 9 of 15, or 11 of 20 such patients develop high-risk, extensive GVHD at any time prior to 18 months, we will consider terminating the trial. The probability of having these many poor outcomes (or more) if the true rate of GVHD is 40% is 0.087, 0.17, 0.10, and 0.13, respectively.

Given the heterogeneity of the patient population included, one cannot formulate expectations regarding overall survival and progression-free survival (PFS). Unless the above described monitoring boundaries are exceeded, the protocol will continue to accrue until 53 patients have been enrolled. At the conclusion of the study, 95% confidence intervals will be generated for PFS, the incidence of graft failure, and the frequency of active and extensive GVHD. Kaplan-Meier estimates of progression-free and overall survival rates will be calculated, both overall and for the good and high risk groups separately, and the median progression-free and overall survival times and their associated 95% confidence intervals derived using the method described in Brookmeyer and Crowley.[26] Descriptive statistics related to the frequency of adverse events and of changes in laboratory values will be generated.

An independent Data and Safety Monitoring Committee will be established to review the data and make recommendations regarding the continuation or discontinuation of the trial.

Addendum: As of March 2010, 34 patients have been accrued to the protocol and no excess graft failure or GVHD have been observed (Rich etal, ASH abstract 3114, 2009). The protocol is being modified for use of the Miltenyi column because of market supply reasons. But the stopping rules no longer apply.
Addendum 2 as of March 2012. We will expand the number of subjects to provide more robust estimates of outcome. The vast majority of subjects received fludarabine, melphalan

conditioning for AML/MDS. Of 73 subjects enrolled so far, 60 have received fludarabine and melphalan reduced intensity. We recently reported our experience with the first 45 subjects receiving reduced intensity fludarabine melphalan (Liu, Blood 2011). Only four of 45 had graft failure (lack of haplo and cord blood engraftment). The stopping rules indicate that if 8 or more of the first 40 have graft failure, the trial would be stopped. Engraftment will continue to be monitoried. A secondary endpoint was GVHD and the rates have been low and below the 40% chronic GVHD considered for stopping the trial. The cumulative incidence of grade II-IV GVHD was 24% and for cGVHD 6 % at one year.

Since the safety of this approach has been established based on low graft failure and low GVHD, overall survival and progression free survival become more of interest. One year overall survival was 55% and progression free-survival 42%. The majority 29 (64%) had AML and MDS. In addition, 21/45 (47%) had high risk disease defined as refractory, untreated relapse. Expanding our sample size will allow us to generate OS and PFS estimates in those by disease risk (high-risk versus standard/low) and for those with AML/MDS. A better estimate of efficacy is essential as future studies are now being planned including a randomized trial. An additional 20 subjects will accrue approximately 87 who will receive fludarabine-melphalan divided into 46 with low to standard risk disease and 41 with high-risk disease (assuming the proportion in the first 45 subjects is maintained). This will also result in enrolling approximately 55 to 56 subjects with AML/MDS and of those, we estimate 30 will have low to standard risk disease. Generating estimates in the cohort of MDS/AML with standard risk disease has emerged as essential as this represents the most common transplant indication.

10.0 DATA AND PROTOCOL MANAGEMENT

- PROTOCOL COMPLIANCE Patients will be reviewed weekly during admission by the study investigators who will score the patient for standard endpoints. After discharge they will be reviewed at least once a month.

- DATA ENTRY Data must be entered into the Protocol Data Management System before a course of therapy can be given. A brief explanation for required but missing data should be recorded as a comment.

- ACCURACY OF DATA COLLECTION The Study Chairman will be the final arbiter of toxicity should a difference of opinion exist.

11.0 CRITERIA FOR REMOVAL FROM PROTOCOL

- At patient request.
- Clinical progression. Such patients may be treated on other treatment protocols or at the investigator's discretion. Such patients will continue to be monitored for end-points relating to engraftment, GVHD and immune reconstitution.

12.0 REPORTING REQUIREMENTS

- Any unexpected, life-threatening and/or serious (grade 3 or 4) toxicity or grade 3 or 4 GVHD will be reported immediately to the Study Chairman. The Chairman will be responsible for notifying the Surveillance Committee.
- **Expected toxicities are those listed in the consent form and include regimen-related toxicities, myelosuppression, opportunistic infections such as CMV reactivation, or GVHD. These will not be routinely reported to the IRB even if they require admission.**
- On the other hand, such toxicities will be monitored by the PI and the transplant team and reported regularly at the High Risk Protocol Committee of the Cancer Center.
- All deaths that are not due to the disease recurrence will be reported to the IRB.

13.0 RETROSPECTIVE REVIEW

As of October 2012, we plan to retrospectively analyze the results of this haplo-cord SCT protocol with the results of Haploidentical stem cell transplant at Peking Institute of Hematology in Beijing. The analysis will focus on the AML/MDS population first. The main purpose of the analysis is to compare the patient characteristics, pre-transplant co-morbidities, and outcomes of transplant from these two centers; including neutrophil, platelet engraftment; progression free survival, overall survival, incidence of acute GVHD, and chronic GVHD et al. In addition, we would like to compare the immune re-constitution after these two types of transplant. The datasets will be shared between the University of Chicago and the Peking institute of Hematology in Beijing without identifiable patient information (coded), each patient will be marked by code without name or medical record number. Both centers have IRB approval for the transplant protocol. The IRB has approved the Peking Institute of Hematology retrospective review.

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APPENDIX A

TREATMENT PLAN

FLUDARABINE – MELPHALAN

Conditioning:

	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0
Fludarabine	30 mg/m^2	30 mg/m^2	30 mg/m^2	30 mg/m^2	30mg/m^2			Haplo
Melphalan					70 mg/m^2	70 mg/m^2		and
rATG	1.5 mg/**kg**		1.5 mg/**kg**		1.5 mg/**kg**		1.5 mg/**kg**	Cord

GVHD Prophylaxis:
Tacrolimus 0.03 mg/kg/day IV CI over 24 hr from 4 PM day -2 until engraftment or when patient is able to take PO, then tacrolimus approximately 0.09 mg/kg PO in 2 divided doses. Tacrolimus should be given at full dose to maintain levels of 5-15 ng/mL through day 180, tapered by 20% every week thereafter. Early tapering can be given for repeated infection, poor chimerism, or high-risk disease.

Additional GVHD prophylaxis according to standard of care.

APPENDIX B

TREATMENT PLAN

FLUDARABINE –THIOTEPA – TBI

Conditioning:

	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0
Fludarabine	30 mg/m^2	30 mg/m^2	30 mg/m^2	30 mg/m^2	30 mg/m^2			Haplo
Thiotepa	5 mg/**kg**	5 mg/**kg**						and
TBI					BID	BID	BID	Cord
rATG	1.5 mg/**kg**		1.5 mg/**kg**		1.5 mg/**kg**		1.5 mg/**kg**	

GVHD Prophylaxis:

Tacrolimus 0.03 mg/kg/day IV CI over 24 hr from 4 PM day -2 until engraftment or when patient is able to take PO, then tacrolimus approximately 0.09 mg/kg PO in 2 divided doses. Tacrolimus should be given at full dose to maintain levels of 5-15 ng/mL through day 180, tapered by 20% every week thereafter. Early tapering can be given for repeated infection, poor chimerism, or high-risk disease.

Additional GVHD prophylaxis according to standard care.

APPENDIX C

TREATMENT PLAN

FLUDARABINE – BUSULFAN

Conditioning:

	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0
Fludarabine	30 mg/m^2	30 mg/m^2	30 mg/m^2	30 mg/m^2	30 mg/m^2			Haplo
Busulfan		130 mg/m^2	130 mg/m^2	130 mg/m^2	130 mg/m^2			and
rATG	1.5 mg/**kg**		1.5 mg/**kg**		1.5 mg/**kg**		1.5 mg/**kg**	Cord

GVHD Prophylaxis:

Tacrolimus 0.03 mg/kg/day IV CI over 24 hr from 4 PM day -2 until engraftment or when patient is able to take PO, then tacrolimus approximately 0.09 mg/kg PO in 2 divided doses. Tacrolimus should be given at full dose to maintain levels of 5-15 ng/mL through day 180, tapered by 20% every week thereafter. Early tapering can be given for repeated infection, poor chimerism, or high-risk disease.

Additional GVHD prophylaxis according to standard care.

APPENDIX D

BACKGROUND DRUG INFORMATION

BUSULFAN

AVAILABILITY
Commercially available in 60 mg/ 10 mL ampuls.

STORAGE & STABILITY
Store ampuls under refrigeration at 2°C to 8°C. The diluted solution is stable for up to 8 hours at room temperature (25°C), but the infusion must also be completed within that 8-hour time frame. Dilution of busulfan injection in 0.9% sodium chloride is stable for up to 12 hours under refrigeration (2°C to 8°C), but the infusion must also be completed within that 12-hour time frame.

PREPARATION
Dilute busulfan injection in 0.9% sodium chloride injection or dextrose 5% in water per institutional pharmacy guidelines.

ADMINISTRATION
Intravenous busulfan should be administered via a central venous catheter per institutional guidelines.

TOXICITY
Severe myelosuppression with marrow ablation, alopecia, and mild nausea/vomiting are expected. Alopecia may not be completely reversible. Liver toxicity, including severe and possibly fatal veno-occlusive disease (<5%), may occur. Pulmonary toxicity is rare. Darkening of the skin may occur and may last several months. Seizures may occur (<5%). Anti-seizure prophylaxis should be administered per transplant guidelines. Benzodiazepines, eg. Clonazepam (Klonopin®) 1 mg PO TID, may be used. Busulfan causes immunosuppression with the risk of opportunistic infection even after resolution of neutropenia. Busulfan is expected to cause nearly universal infertility in the doses used, although men may occasionally father children. Grade III mucosities, diarrhea, and abdominal pain, hemorrhagic cystitis, "hand-foot syndrome" with neuropathic pain has also been reported.

FILGRASTIM (G-CSF: Granulocyte Colony Stimulating Factor, Neupogen®)

AVAILABILITY
G-CSF is commercially available in 1.0 and 1.6 mL vials containing 300 mcg and 480 mcg G-CSF, and in prefilled syringes containing 300 mcg/0.5 mL and 480 mcg/0.8 mL.

STORAGE & STABILITY

Intact vials and prefilled syringes should be stored under refrigeration. Do not allow the drug to freeze.

ADMINISTRATION

The daily dose of G-CSF should be injected subcutaneously in one or two sites. The dose following peripheral blood stem cell infusion is 5 mcg/kg/day. The dose of G-CSF may be rounded up to the nearest vial size.

TOXICITY
The most common side effect associated with G-CSF is bone pain. Bone pain is usually reported as mild or moderate and, if necessary, may be treated with non-opiod or opiod analgesics.

FLUDARABINE (Fludara®)

AVAILABILITY
Fludarabine is commercially available as a white, lyophilized powder. Each vial contains 50 mg of fludarabine, 50 mg of mannitol and sodium hydroxide to adjust pH.

STORAGE & STABILITY
Intact vials should be stored under refrigeration. Reconstituted vials are stable for 16 days at room temperature or under refrigeration. Solutions diluted in D_5W or NS are stable for 48 hours at room temperature or under refrigeration.

PREPARATION
Fludarabine should be reconstituted with Sterile Water for Injection, USP or normal saline per institutional pharmacy guidelines.

ADMINISTRATION
Fludarabine will be administered as an IV infusion over 30 minutes.

TOXICITY
Myelosuppression, (dose-limiting toxicity), fever, mild nausea and/or vomiting, diarrhea, stomatitis, skin rashes, myalgia, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, autoimmune hemolytic anemia (may be life-threatening), peripheral neuropathy, and pulmonary toxicity. (Both pneumonia and hypersensitivity reactions have been reported. Fatal pulmonary toxicity has been described, especially when fludarabine was used in combination with pentostatin. Severe, fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status was encountered almost exclusively after very high doses of fludarabine. Such toxicity has only been rarely demonstrated at the 25-30 mg /m^2 dosage of fludarabine. Very rarely described complications include transfusion-associated graft versus host disease. Tumor lysis syndrome has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed.

MELPHALAN (Alkeran®)

AVAILABILITY
Melphalan for IV use is commercially available in sterile 50 mg vials. The product is a lyophilized powder with 20 mg povidone per vial. Also provided is 10 mL of special diluent for use in reconstituting the product. The special diluent has 0.20 g sodium citrate, 6 mL propylene glycol, 0.5 mL 95% ethanol, and sterile water.

STORAGE & STABILITY
Intact vials should be stored at room temperature (15°C-30°C) and protected from light. Reconstituted solutions are chemically and physically stable for at least 90 minutes at room temperature. Solutions further diluted in 0.9% sodium chloride to a concentration of 0.1 mg/mL to 0.45 mg/mL are stable for at least 60 minutes. Solutions diluted to 1 mg/mL are reported to be physically stable for at least 4 hours at room temperature-chemical stability of this dilution is not known. Because of the relative instability of melphalan solutions, it is recommended that administration of the diluted solution be completed within 60 minutes of reconstitution. Reconstituted solutions should not be refrigerated.

PREPARATION
Melphalan should be prepared immediately before intended use. Each vial is reconstituted with 10 mL of the special diluent to yield a concentration of 5 mg/mL. The reconstituted solution may be diluted with 0.9% sodium chloride to a concentration of 0.1 mg/mL to 0.45 mg/mL.

ADMINISTRATION
The total dose of melphalan will be administered by short IV infusion over 30-60 minutes, as per institutional pharmacy guidelines.

TOXICITY
The major toxicity of melphalan is bone marrow suppression, usually lasting four to eight weeks. Other toxicities include nausea, vomiting, diarrhea, and mucositis. Less common toxicities include pulmonary fibrosis, interstitial pneumonitis, vasculitis, alopecia, hemolytic anemia, and allergic reactions. Transient rises in BUN and creatinine have occurred with high dose melphalan and also acute renal failure. Tissue necrosis may result if infiltration occurs.

MYCOPHENOLATE MOFETIL (Cellcept®; Myfortic®; MMF)

AVAILABILITY
Mycophenolate mofetil is available as a **Capsule**, as mofetil: CellCept®: 250 mg;
as **Injection**, powder for reconstitution, as mofetil hydrochloride: CellCept®: 500 mg [contains polysorbate 80]; as **Powder** for oral suspension, as mofetil: CellCept®: 200 mg/mL (225 mL) [provides 175 mL suspension following reconstitution; contains phenylalanine 0.56 mg/mL; mixed fruit flavor]; as a **Tablet**, as mofetil: CellCept®: 500 mg [may contain ethyl alcohol]; and as a **Tablet, delayed release**, as mycophenolic acid: Myfortic®: 180 mg, 360 mg [formulated as a sodium salt].

STORAGE & STABILITY
Intact vials should be stored at room temperature 15ºC to 30ºC (59ºF to 86ºF). Store solutions at 15ºC to 30ºC (59ºF to 86ºF) and begin infusion within 4 hours of reconstitution. Store capsules at room temperature of 15ºC to 39ºC (59ºF to 86ºF). Tablets should be stored at room temperature of 15ºC to 39ºC (59ºF to 86ºF) and protected from light. Store powder for oral suspension at room temperature of 15ºC to 39ºC (59ºF to 86ºF). Once reconstituted, the oral solution may be stored at room temperature or under refrigeration. Do not freeze. The mixed suspension is stable for 60 days.

PREPARATION
Mycophenolate mofetil is stable in D5W should be reconstituted per institutional pharmacy guidelines.

ADMINISTRATION
Intravenous solutions of mycophenolate mofetil should be administered over at least 2 hours (either peripheral or central vein); do not administer intravenous solution by rapid or bolus injection. Oral dosage formulations (tablet, capsule, suspension) should be administered on an empty stomach to avoid variability in MPA absorption. The oral solution may be administered via a nasogastric tube (minimum 8 French, 1.7 mm interior diameter); oral suspension should not be mixed with other medications. Delayed release tablets should not be crushed, cut, or chewed.

TOXICITY
Pain, abdominal pain, fever, headache, infection, sepsis, asthenia, chest pain, back pain, hypertension, tremor, insomnia, dizziness, acne, rash, diarrhea, constipation, mild N/V, oral monoliasis, anemia, leukopenia, thrombocytopenia, hypochromic anemia, leukocytosis, peripheral edema, hypercholesterolemia, hypophosphatemia, edema, hypo or hyperkalemia, hyperglycemia, infection, dyspnea, cough increase, pharyngitis, bronchitis, pneumonia, UTI, hematuria, kidney tubular necrosis, urinary tract disorder.

RABBIT ANTITHYMOCYTE GLOBULIN (Thymoglobulin®**,** rATG)

AVAILABILITY
Antithymocyte globulin is commercially available. Each package contains two vials: the first vial contains 25 mg antithymocyte globulin, and the second vial contains > 5 mL SWFI diluent.

STORAGE & STABILITY
Ampuls must be refrigerated (2^{o}C-8^{o}C/ 36^{o}F-46^{o}F),). Do not freeze.

PREPARATION
Reconstitute 25 mg vial with diluent provided by manufacturer (SWFI > 5 mL). Roll vial gently to dissolve powder. Use contents of vial within 4 hours of reconstitution. Dilute dosage to a final concentration of 0.5 mg/mL in 0.9% sodium chloride injection or 5% dextrose injection. Gently invert admixture 1-2 times to mix solution. Use admixture solution immediately. Final concentration must be 0.5 mg/mL.

ADMINISTRATION
Infuse the first dose over at least six hours, and subsequent doses over at least 4 hours. Infuse through a 0.22 micron in-line filter into a high-flow vein. Premedications include acetaminophen 650 mg PO, diphenhydramine 25-50 mg PO/IV, and methylprednisolone 1 mg/kg (at the initiation and half-way through antithymocyte globulin administration).

TOXICITY
Infusion-related toxicities, including fevers, chills, rash, dyspnea, cardiovascular (hypo- or hypertension, tachycardia, edema, chest pain). In rare cases, anaphylaxis has been reported in which case the infusion should be terminated immediately, and emergency treatment with epinephrine and other resuscitative measures should be instituted. rATG should not be administered again to this patient. Immunosuppression is a common feature of rATG and can result in severe infections including sepsis, CMV, and urinary tract infections. Serum sickness, neutropenia (57%), thrombocytopenia (37%), leucopenia (57%), pain (46%), headache (40%), nausea and diarrhea (37%), peripheral edema (34%), systemic infection, malaise, pain, stomatitis, GI bleed, swelling or redness at injection site, myalgia, back pain, development of human anti-rabbit antibodies (HARA).

TACROLIMUS (Prograf®, FK506)

AVAILABILITY
Tacrolimus is commercially available as an injection (5 mg/mL; 1 mL ampuls) and as oral capsules (0.5 mg, 1 mg, and 5 mg).

STORAGE & STABILITY
Store tacrolimus capsules and injection at controlled room temperature, 15°C-30°C (59°F-86°F).

PREPARATION – FOR IV USE
Tacrolimus injection must be diluted prior to IV infusion with 0.9% sodium chloride or 5% dextrose injection to a concentration of 4-20 mcg/mL. Solutions should be prepared in non-PVC plastic or glass. Tacrolimus injection and diluted solutions of the drug should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit.

ADMINISTRATION
Oral therapy should be started as soon as possible as per protocol and 8 to 12 hours after stopping intravenous therapy. Oral doses will be administered twice a day. The conversion from IV to oral therapy should take into account concomitant medications (such as voriconazole).

TOXICITY
In patients receiving tacrolimus, 5% to 47% experienced anemia, 8% to 32% experienced leukocytosis, and 14% to 24% experienced thrombocytopenia. Rare cases of microangiopathic hemolytic anemia have been reported. Chest pain was reported in 19%. Mild to moderate hypertension is a common adverse effect associated with tacrolimus therapy. Antihypertensive therapy may be required. The most common adverse effects of tacrolimus have involved the central nervous system, and include headache (37% to 64%), tremors (48% to 56%), insomnia (32% to 64%), paresthesia (17% to 40%), and dizziness (19%). Tremor and headache may respond to dosage reduction. Visual changes, agitation, anxiety, confusion, seizures, depression, hallucinations, myoclonus, neuropathy, psychosis, incoordination, and abnormal dreams have been reported in 3% to 15%. Hyperkalemia (13% to 45%), hypokalemia (13% to 29%) hypophosphatemia (49%) and hypomagnesemia (16% to 48%) have been associated with tacrolimus therapy. Hyperuricemia has been reported in >3%. Gastrointestinal adverse effects included nausea (32% to 46%), vomiting (14% to 29%), anorexia (7% to 34%), constipation (23% to 35%), and diarrhea (37% to 72%). Nephrotoxicity was reported in 38% to 52% of liver and kidney transplant patients, respectively. Hematuria has been reported in greater than 3%. Abnormal liver function tests have been reported in 6% to 36% of patients; ascites in 7% to 27%.

Other effects reported in clinical trials include pain, fever, asthenia, back pain, and peripheral edema. The incidence of hyperglycemia was 17% and may require therapy with insulin. Other less frequently occurring effects include abscess, chills, peritonitis, and photosensitivity reactions. Anaphylaxis has been reported in a few patients receiving intravenous tacrolimus. Tacrolimus injection contains cremophor which in other drugs has been associated with anaphylaxis. Because tacrolimus is an immunosuppressant, the risk of opportunistic infections is increased.

DRUG INTERACTIONS
Tacrolimus is metabolized by cytochrome P450 3A4. Drugs that are inhibitors (e.g. itraconazole) of inducers (e.g. phenytoin) of 3A4 might be expected to increase or decrease tacrolimus concentrations, respectively, possibly resulting in increased or decreased effects.

THIOTEPA (Thioplex®)

AVAILABILITY
Thiotepa is commercially available.

STORAGE & STABILITY
Store intact vials under refrigeration (2°C-8°C) and protect from light. **Not stable** at room temperature for any duration of time.

PREPARATION
Dilute powder 1.5 mL SWI to a concentration of 10.4 mg/mL which is stable for 8 hours at refrigeration. Further dilutions in NS per institutional pharmacy guidelines.

ADMINISTRATION
Thiotepa will be administered as an IV infusion over 2 hours per institutional guidelines.

TOXICITY
Myelosuppression, anorexia, anaphylaxis, hyperuricemia. High dose thiotepa: Severe myelosuppression, severe N/V, stomatitis. LFT abnormalities, hyperpigmentation, confusion. Intrathecal thiotepa: Headache, nausea and vomiting. May require dose reduction in renal impairment.

APPENDIX E

ZUBROD (ECOG) SCALE OF PERFORMANCE STATUS

Zubrod Status

0 = No symptoms

1 = Symptoms, fully ambulatory

2 = Requires nursing assistance or equivalent; bedridden < 50% or normal day.

3 = Bedridden > 50% or normal day

4 = Bedfast

APPENDIX F

PRZEPIORKA CRITERIA FOR ACUTE GVHD

Consensus Criteria for Grading of Acute GVHD

	Skin		*Liver*		*Gut*
Stage					
1	Rash <25%[a]		Bilirubin 2-3 mg/dL[b]		Diarrhea >500 mL/d[c] or persistent nausea[d]
2	Rash 25-50%		Bilirubin 3-6 mg/dL		Diarrhea >1000 mL/d
3	Rash >50%		Bilirubin 6-15 mg/dL		Diarrhea >1500 mL/d
4	Generalized erythroderma with bullae		Bilirubin >15 mg/dL		Severe abdominal pain with or without ileus
Grade[e]					
I	Stage 1-2		None		None
II	Stage 3	or	Stage 1	or	Stage 1
III	----		Stage 2-3	or	Stage 2-4
IV	Stage 4	or	Stage 4	or	Stage 4

[a]Use "Rule of Nines" or burn chart to determine extent of rash.
[b]Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented.
[c]Volume of diarrhea applies to adults. For pediatric patients, the volume of diarrhea should be based on body surface area. Downgrade one stage if an additional cause of diarrhea has been documented.
[d]Persistent nausea with histologic evidence of GVHD in the stomach or duodenum.
[e]Criteria for grading given as degree of organ involvement required to confer that grade.
[f]Grade IV may also include lesser organ involvement when the Karnofsky performance status is <50%, so patients with Stage 4 gut GVHD are usually grade IV.

APPENDIX G
HAPLO-CORD SAMPLES

Blood Samples are drawn:
Before conditioning (plus bone marrow sample)
Day 0
Day 7
Day 10
Day 14
Day 28 (plus bone marrow sample)
Day 100 (plus bone marrow sample)
Day 180 (plus bone marrow sample)
At relapse (plus bone marrow sample)
One year and yearly thereafter (plus bone marrow sample)

Blood samples are ordered on a **miscellaneous requisition** -
Fill in the **top** portion:
Lab: "Kovler – Jodie"
Specimen: "Blood"
Examination: "Haplo-cord study samples"

On the **bottom right**, add:
"Haplo-cord study samples" and **date** of sample

On the **left**, give these instructions:
"Please draw 4 green top and 1 red top tube of peripheral blood (PB).
Send to Apheresis, tube station #663, and call Jodie at 58127, or pager 9950, for pickup".

Bone Marrow Samples:
Bone marrow exams are performed as above (pre, days 28, 100, 180, relapse, 1 year):

On the **bone marrow requisition**, request in the "**Other** Section":
"Please draw 2 green top tubes of BM aspirate. Send to Apheresis, tube station #663, and call Jodie at 58127, or pager 9950, for pick up"

If the "Other Section" is used, you may also request these bone marrow samples on a miscellaneous requisition and attach to the bone marrow requisition. In the **Specimen** field of the miscellaneous requisition, write "bone marrow aspirate". And in the **lower right** add: "Please draw 2 green top tubes of BM aspirate. Send to Apheresis, tube station #663, and call Jodie at 58127, or pager 9950, for pick up"

Order Cytogenetics **FISH** analysis if either the haploidentical or cord donor is a **sex mismatch**.

Additional Information:
Peripheral blood VNTR (chimerism) will be performed every two weeks for the first 100 days following a Haplo-Cord transplant (on days 14, 28, 42, 56, 70, 84, 100).

If any questions, please call:
Scott Allen – 21613, or pager 9377. Or contact Study Chair Dr. Andrew Artz

APPENDIX H

INFUSION OF CRYOPRESERVED HEMATOPOIETIC PROGENITOR CELLS BY NURSES

SEE ATTACHED POLICY: 431.02
University of Chicago Medical Center
Progenitor Cell Transplantation & Cell Therapy Program

APPENDIX I:
ALLO DONOR SELECTION CRITERIA

SEE ATTACHED POLICY: 051.4
University of Chicago Medical Center
Progenitor Cell Transplantation & Cell Therapy Program

APPENDIX J:
APHERESIS EVALUATION AND MANAGEMENT

SEE ATTACHED POLICY: 410.03
University of Chicago Medical Center
Progenitor Cell Transplantation & Cell Therapy Program