A Phase II Study of Parathyroid Hormone following Myeloablative Sequential Unrelated Cord Blood Transplantation

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**Principal Investigator:** Karen K. Ballen, MD

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I. Title: A Phase II Study of Parathyroid Hormone Following Myeloablative Sequential Unrelated Cord Blood Transplantation

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II. Background and Rationale

A. Clinical Experience with BMT

Myeloablative chemotherapy or chemoradiotherapy and allogeneic stem cell transplantation is an accepted curative therapy for many cancers, leukemias, .and genetic disorders 1-3. Long-term disease free survival probabilities of up to 70-80% can be achieved, particularly in young patients with matched donors 4. Results of unrelated donor transplantation range from 20-30% for high-risk patients to 60% for young patients with chronic myelogenous leukemia 5,6.

Given the size of most American families, only 30% of patients will have a matched sibling donor 7. The Anthony Nolan Registry, the Caitlin Raymond International Registry, the National Marrow Donor Program (NMDP), and other international registries were established to provide a source of volunteer marrow donors for patients without family donors 8. Although these registries have grown to include over four million volunteer bone marrow donors, approximately 50% of patients are unable to find a suitably matched unrelated marrow donor and proceed to transplant in a timely fashion. It is particularly difficult for African Americans and other minorities to find matched unrelated bone marrow donors 9.

Placental or umbilical cord blood (CB) has been shown to contain sufficient progenitor cells to provide durable engraftment 10. Repositories of unrelated cord blood collected from volunteer donors just after delivery have been established in the United States and Europe 11-16. An estimated 70,000 cord blood units are available worldwide, and can be shipped for immediate use 17.

The first related cord blood transplant was performed in 1988, in a child with Fanconi’s Anemia 18. The clinical experience with cord blood transplantation has recently been reviewed 19.
The largest single institution experience is at Duke University. Rubinstein et al reported on the results of 562 cord blood transplants facilitated by the New York Blood Center. Younger age and a high nucleated cell (NC) dose/kg were associated with improved survival. Eurocord data suggests a 30% disease-free survival in pediatric recipients of unrelated cord blood transplants. The nucleated cell dose/kg infused correlated with engraftment and survival. Engraftment was improved with a stem cell dose greater than 3.7 x 10^7 NC/kg infused. The average nucleated cell dose of the cord blood units in many cord blood banks is 10 x 10^8 NC. Therefore, most single cord blood units are only acceptable for children and small adults. The CD34 dose may also be important for engraftment and survival. There have been several attempts at cord blood expansion, but the optimal methods have not been defined.

Laughlin and colleagues have reported that single unrelated cord blood units could restore hematopoiesis in adults after myeloablative transplant conditioning. Sixty-eight patients, ages 18-55, median weight 69 kg, received a cord blood stem cell transplant. Sixty percent of patients developed Grades II-IV GVHD and 20% of patients developed Grades III-IV GVHD. Eighteen patients survive forty months after transplant. The median number of nucleated cells, prior to freezing and at thawing, was 2.1 x 10^7 NC/kg and 1.6 x 10^7 NC/kg respectively. The 100-day transplant related mortality was 41%.

Sanz et al have reported on 22 adult recipients of a cord blood transplant in Spain. The median age was 29 years and all patients had hematologic malignancies and received an ablative non-TBI conditioning regimen. One patient received an HLA matched 6/6 graft, 13 patients received a 5/6 matched graft, and 8 patients received a 4/6 graft. Median number of nucleated cells/kg infused was 1.7 x 10^7. Disease-free survival at one year was 53%.

The Eurocord group has reported on 149 adults who underwent transplantation of cord blood from unrelated donors. Transplantation related mortality at 180 days was 56% in adult recipients, as compared to 32% in 366 pediatric recipients. The nucleated cell count/kg infused correlated with survival; patients who received less than 1 x 10^7 NC/kg had a 75% probability of death; patients who received more than 3 x 10^7 NC/kg had a 30% probability of death.

The Japanese group has just published impressive data comparing 68 adult unrelated cord blood recipients with 45 adult unrelated bone marrow recipients. Ninety-four percent of patients received a cell dose of > 2.0 x 10^7 NC/kg. The transplant related mortality was 9% for cord blood recipients and 29% for unrelated bone marrow recipients. The two-year disease free survival was 74% for cord blood and 44% for unrelated bone marrow. These results are superior to those reported in the American and European series, perhaps due to the smaller sample size and genetic homogeneity of this population. A recent retrospective study from the International Bone Marrow Transplant Registry compared survival in adults with leukemia receiving unrelated bone marrow versus unrelated cord blood. Deaths related to infection were highest in the cord blood recipients, but acute GVHD was
less likely in the cord blood group, compared to the mismatched bone marrow cohort. Three-year leukemia free survival was 33% for HLA matched bone marrow, 23% for cord blood, and 19% for one antigen mismatched bone marrow.29

The ability to transplant adults and larger children with cord blood would provide another stem cell transplant option for patients that do not have a matched related or unrelated donor. In addition, cord blood transplants may also provide a readily available supply of stem cells, and reduced incidence of graft versus host disease.

B. Preliminary Clinical Experience with Sequential Cord Blood Transplants

The relatively low number of hematopoietic stem cells in cord blood units has limited the success in adult patients, and has triggered investigation into the use of multiple, sequentially administered cord blood units. An early report of patients infused with multiple, mismatched units suggested that crossed immunologic rejection would not occur.30

Recent studies of adult recipients have evaluated the outcome of transplanting two cord blood units to adult recipients, thereby increasing the number of stem cells infused. The Minnesota group has treated adult recipients with one or two cord blood units to achieve a minimum infusion of $3.5 \times 10^7$ NC/kg.31 Patients receive a non-myeloablative conditioning regimen. Cord blood units were a 4/6 HLA match with the patient and with each other. Twenty-one patients received conditioning with busulfan/fludarabine/low-dose TBI and 22 patients received cyclophosphamide/fludarabine/low dose TBI. Cyclosporine and mycophenolate mofetil were used for GVHD prophylaxis. Twenty-four of 43 patients received two cord blood units. HLA typing was performed at the antigen level for Class I and the allele level for Class II. The incidence of engraftment was 76% for the first regimen and 94% for the second regimen; the median days to an absolute neutrophil count of 500 were 26 days for the first regimen and 10 days for the second regimen. The risk of Grades III or IV acute GVHD was 9%. One hundred day TRM was 48% for the busulfan/fludarabine/TBI group and 28% for cyclophosphamide/fludarabine/TBI group. Death was due mainly to organ failure and infection. Overall survival at one year was 39%. One cord unit predominated by Day +100. The factors which determine which cord unit predominates are not clear, as it is not necessarily the bigger unit or the closer HLA match.

The double cord blood approach has also been used after myeloablative conditioning regimen.32 A myeloablative regimen was chosen for these patients due to high risk disease. Twenty-three patients received two unrelated umbilical cord blood units after cyclophosphamide 60 mg/kg Days –7, -6/total body irradiation conditioning regimen 1320cGy in 8 fractions. Twenty-one patients also received fludarabine 25mg/m2 Days –8, -7, -6. Graft versus host disease prophylaxis was cyclosporine/mycophenolate mofetil (MMF). Neutrophil engraftment occurred at a
median of 23 days. The incidence of Grades II-IV GVHD was 65% and the incidence of Grades III-IV GVHD was 13%. One cord unit predominated in all patients by Day +100. With a median follow up of 10 months, the predicted one-year survival was 57%.

A Phase I study of sequential cord blood transplantation using the nonmyeloablative conditioning of fludarabine/melphalan/thymoglobulin has completed accrual at Massachusetts General Hospital and the Dana Farber Cancer Institute. The conditioning regimen was fludarabine 30mg/m²/day x 6 days, melphalan 100mg/m²/day x 1 day, and rabbit antithymocyte globulin 1.5 mg/kg/day x 4 days. GVHD prophylaxis was cyclosporine and mycophenolate mofetil (MMF). Cord units were a 4/6 HLA match or better with each other and with the patient and achieved a minimum pre-cryopreservation cell dose of 3.7 x 10⁹ nucleated cells/kg.

Twenty-one patients, median age 49 years, were treated. The diagnoses were AML (n=8), ALL (n=1), NHL (n=5), CLL (n=2), MDS (n=1), Hodgkins Disease (n=2), and aplastic anemia (n=2). Nineteen percent of patients were non-Caucasian. The combined cell dose infused was a median of 4.0 x10⁷ NC/kg (range 2.9-5.1 x 10⁷). The median time to an absolute neutrophil count > 0.5 x 10⁹/L was 20 days and the median time to an unsupported platelet count >20 x 10⁹/L was 41 days. Two patients, both with MDS complicating aplastic anemia, experienced primary graft failure and underwent a second cord blood transplant. The two patients with graft failure had not received prior chemotherapy, suggesting that patients with a relatively intact immune system are at higher risk of rejection after a nonmyeloablative procedure and might do better with an ablative regimen.

Acute graft vs. host disease Grades II-IV was seen in 26% of patients. There was one patient with Grade III GVHD and no patients with Grade IV GVHD. There were no deaths from GVHD. The 100-day transplant related mortality was 14%. The deaths were due to a CNS bleed, Epstein Barr virus lymphoproliferative disorder, and staphylococcal sepsis. There were two deaths after Day +100. One patient died from chronic GVHD and vancomycin resistant enterococci sepsis, and another patient had aspergillus infection after having received a second double cord blood transplant. Chimerism analysis showed predominance of one cord by Day +100 in all evaluable patients. In 76% of patients, the predominant cord was the first cord blood unit infused. With a median follow up of 11 months, the one-year disease free survival is 70%.

C. Experience with Ablative Conditioning Regimens

The optimal conditioning regimen for cord blood, and other stem cell transplants, is unknown. Historically, transplant was limited to younger patients without other medical conditions due to the risk of regimen related end organ toxicity and graft
versus host disease. While nonmyeloablative approaches may be appropriate for older patients and those patients with slower growing malignancies, ablative transplantation regimens remain the choice for younger patients, particularly those with high risk diseases.  

The regimen chosen for this study, cyclophosphamide and total body irradiation, is a standard ablative transplant conditioning regimen, in use for over 16 years. Total body radiation is thought to be preferable in diseases with a high likelihood of CNS recurrence, such as acute lymphoblastic leukemia and high grade lymphomas. An ablative regimen may be most appropriate for those patients who have a lower rate of engraftment after nonmyeloablative cord blood transplant, particularly those patients with untreated aplastic anemia, myelodysplasia, and chronic myelogeneous leukemia. Fludarabine is added here, as used in Minnesota, because of its profound ability to deplete the CD4+ T cells that are implicated in graft rejection.

D. Selection of GVHD Prophylaxis Regimen

The GVHD prophylaxis regimens used in many cord blood pediatric transplant protocols has been either cyclosporine or tacrolimus combined with prednisone. Methotrexate has been avoided because of concern over the already delayed engraftment. In adult cord blood protocols, steroids have been eliminated because of the concern over infection. A common GVHD prophylactic regimen is cyclosporine/MMF used by the Minnesota group and in our Phase I study above. In this study, tacrolimus is substituted for cyclosporine due to the favorable toxicity profile of tacrolimus and MMF.

E. Use of Parathyroid Hormone

Despite advances in cord blood transplantation using cord blood units with higher cell doses and the use of two cord blood transplants, delayed engraftment (particularly platelet engraftment) and poor immune reconstitution remain major causes of morbidity and mortality following cord blood transplantation.

An approach to improving these results is to manipulate the stem cell niche. Osteoblasts produce hematopoietic growth factors and are activated by parathyroid hormone (PTH) or the PTH related protein, through the PTH/PTHrp receptor (PPR).

The Notch signaling pathway regulates a wide variety of systems, including hematopoietic stem cell self-renewal. The Notch ligand Jagged 1 is expressed by marrow stromal cells and murine osteoblasts. In the murine model, PPR activation in the osteoblasts increases production of Jagged 1, which in turn, activates Notch, resulting in expansion of the stem cell compartment.
To test whether PPR stimulation could have a meaningful physiologic effect, PTH was administered to mice undergoing myeloablative bone marrow transplantation using limiting numbers of donor cells to mimic a setting of therapeutic need. Survival at 28 days in control mice receiving mock injections after transplant was 27%. In contrast, animals receiving pulse dosing of PTH had improved outcomes with 100% survival.41

These studies demonstrate that the osteoblast is a key regulator of the stem cell niche and that PTH is capable of increasing stem cell numbers in vivo. Parathyroid hormone is produced in the parathyroid gland and acts on bone and kidney to maintain calcium levels. Human parathyroid hormone is clinically available and FDA approved for the treatment of osteoporosis in both men and women.42

The dose used was 40 mcg sq daily of PTH 1-34 in the study on men; 4% of the men had elevated calcium levels, but none above 11.5 mg/dl. The dose used in the women was 100 mcg PTH 1-84 daily. Other reported side effects include headache, nausea, joint pain, muscle aches, and frequent urination. A recent study of 126 women using PTH1-34 daily at a dose of 25 mcg and alendronate for the treatment of osteoporosis reported the side effects of muscle aches, redness at injection site, nausea, fatigue, and an elevated urine calcium: creatinine ratio without an elevated serum calcium.45

A Phase I study of Parathyroid Hormone in Addition to G-CSF for Patients requiring Additional Stem Cell Mobilization is currently accruing patients at Massachusetts General Hospital, Dana Farber Cancer Institute, Beth Israel Deaconess Medical Center and MD Anderson Cancer Center.46 This study looks at the safety of PTH in 4 different dosing levels (40mcg, 60 mcg, 80 mcg, and 100 mcg) when given to patients who have failed one or two stem cell mobilization attempts. Patient received 14 days of PTH, and G-CSF for the last four days.

Twenty patients have enrolled to date, and nineteen have completed the two-week treatment period. This study is now enrolling patients on the fourth and final cohort. There has been no dose limiting toxicity (elevated calcium level, low phosphate level, and hypotension). There have been mild side effects such as fever, chills and pain at the site of injection. Seven of fourteen patients who failed one prior mobilization have had an adequate stem cell mobilization (defined as peripheral blood CD34+ count>5/ul) after receiving PTH. One of five patients who had failed two prior mobilization attempts had an adequate stem cell mobilization after receiving PTH.

F. Rationale for Current Study

In this study, we extend our experience with sequential cord blood transplantation. We use a well known myeloablative regimen of fludarabine, cyclophosphamide and total body irradiation to treat those patients who are likely to benefit from an ablative regimen. Tacrolimus will be combined with MMF for the GVHD prophylaxis
regimen. Parathyroid Hormone is added to this regimen in an attempt to improve engraftment. Parathyroid hormone is an approved drug with minimal side effects in the osteoporosis population and this dose of PTH has been determined from a Phase I study in patients with hematologic malignancy.

There is some overlap in eligibility criteria for this study and for Protocol 05-154. “A Phase II study of Tacrolimus and Sirolimus as Graft versus Host Disease Prophylaxis after Sequential Unrelated Cord Blood Transplantation,” which uses a non-myeloablative regimen. The suitability of patients for either protocol will be at the discretion of the treating investigator. In general, younger patients, those patients with more aggressive myeloid malignancies (such as acute leukemia in CR2 or greater with short disease-free interval) and those patients with diseases that may be more likely to experience graft failure after nonmyeloablative regimens (such as aplastic anemia, untreated myelodysplasia) may be more appropriate for this study with an ablative conditioning regimen.

III. Objectives

The Purpose of this study is to determine the days to neutrophil engraftment in patients receiving parathyroid hormone following unrelated sequential (double) cord blood transplantation for patients who have diseases that are capable of being cured by allogeneic stem cell transplant but do not have a matched family or volunteer unrelated donor.

A. Primary Objective

1. To evaluate the days to neutrophil engraftment defined as the first of three consecutive days of an absolute neutrophil count ≥ 500.

B. Secondary Objectives

1. To evaluate the 100 day transplant related (non relapse) mortality
2. To evaluate the days to platelet engraftment (platelet count >20K unsupported)
3. To evaluate the risk of acute and chronic graft versus host disease
4. To evaluate percent donor chimerism--contribution of each cord unit
5. To evaluate relapse rate
6. To evaluate overall and disease free survival
7. To evaluate transfusion support (number of red blood cells and platelet transfusions needed prior to red blood cell and platelet engraftment
IV. Patient Selection (all required tests must be performed within 30 days of registration or 42 days prior to admission for transplantation).

A. Inclusion criteria for eligibility include:

1. Disease criteria:
   a. CML accelerated phase or second stable phase. Patients in first chronic phase are eligible if they have resistance to imatinib.
   b. Myelodysplasia
   c. Aplastic Anemia, not responding to immunosuppressive therapy
   d. Myelofibrosis, either primary or secondary to polycythemia vera
   e. Relapsed lymphoma or Hodgkin’s disease.
   f. Stage III/IV CLL, relapsed after or refractory to at least one fludarabine containing regimen.
   g. AML or ALL in CR 2 or greater or CR 1 with high risk features
2. Age 18-45 years.
3. No prior autologous stem cell transplant.
4. ECOG Performance status of ≤2.
5. Lack of 6/6 or 5/6 matched related donor or lack of 10/10 matched unrelated donor, or a donor is not available in time frame to perform a potentially curative stem cell transplant.
6. DLCO >50% predicted.
7. LVEF > 50% predicted.
8. Calcium <10.5 mg/dl, phosphate > 1.6 mg/dl.

B. Exclusion criteria for eligibility include:

1. Cardiac disease: symptomatic congestive heart failure or RVG or echocardiogram determined left ventricular ejection fraction of < 50%, active angina pectoris, or uncontrolled hypertension.
2. Pulmonary disease: severe chronic obstructive lung disease, or symptomatic restrictive lung disease, or corrected DLCO of < 50% of predicted.
3. Renal disease: serum creatinine > 2.0 mg/dl.
4. Hepatic disease: serum bilirubin > 2.0 mg/dl (except in the case of Gilbert’s syndrome or hemolytic anemia in which the bilirubin can be elevated greater than 2.0mg/dl), SGOT or SGPT > 3 x upper limit normal.
5. Neurologic disease: symptomatic leukoencephalopathy, active CNS malignancy or other neuropsychiatric abnormalities believed to preclude transplantation (previous CNS malignancy, presently in CR is not exclusion).
6. HIV antibody.
7. Uncontrolled infection.
V. Donor Selection

Cord blood donors are enrolled by a number of cord blood banks such as the American Red Cross Cord Blood Program, the New York Blood Center, NETCORD, etc. Donors undergo a strict evaluation and are consented by the cord blood bank. Enrollment and consent of cord blood donors are not covered in this protocol.

A. Selection of Cord Blood Units:

1. The patient and the cord blood units must be a 4/6 HLA A, B, DRB1 match or greater match with each other and with the patient. HLA C and DQ will be tested but will not be used in the match strategy.

2. Determination of histocompatibility will be made by molecular typing of Class I and Class II alleles. All cord blood units will have confirmatory HLA typing performed. The confirmatory HLA typing may be performed at an ASHI accredited laboratory of the cord blood bank or at MGH/Dana Farber/Beth Israel Deaconess.

3. Total combined nucleated cell dose from the 2 cord blood units > 3.7 x 10^7 NC/kg (pre-freeze dose). Each single cord blood unit cell dose must be > 1.5 x 10^7 NC/kg.

B. Choice of cord blood units, when multiple suitable units are available:

1. Cell dose (higher nucleated count and CD 34 cell dose preferable)
2. HLA type (closer A,B DR match; DR match takes preference)
3. Age of unit (fresher unit preferable)

VI. Evaluation, Counseling and Consent of Patient

Patients are referred here for consideration of stem cell transplantation. Patients are completely evaluated and presented at a group conference where the transplant team will decide on the course of treatment. The patient evaluation includes a thorough history and physical examination and a series of studies to confirm medical eligibility. In addition, the patient will undergo a psychological evaluation (by the BMT social worker and/or psychiatrist) prior to transplantation, if indicated by the transplant attending. Financial aspects of the transplant will also be discussed with the patient and his/her family before transplant. Treatment recommendations are then discussed thoroughly with patient and family. The cord blood transplant procedures as well as alternative forms of therapy, as far as they exist, are presented as objectively as possible. The risks and hazards of the procedure are explained to the patient and family. It will be pointed out specifically that the use of parathyroid hormone after cord blood transplantation is experimental. The additional risks of sequential cord blood transplant may include a higher risk of graft versus host disease, graft vs graft disease, and graft rejection. The use of PTH may decrease the time to
neutrophil recovery and the risk of infection, but could potentially increase graft vs host disease. Consent is obtained using forms approved by the Dana Farber Institutional Review Board.

VII. Treatment Plan

A. Identification and Preparation of the Cord Blood Unit

Cord units will be identified through existing cord blood registries such as the American Red Cross, National Marrow Donor Program, New York Blood Center, etc. All cord blood units will have confirmatory HLA typing performed by molecular methods. Cord blood units will be requested and stored in liquid nitrogen vapor until thaw. Cord blood units must be present on site and accepted by processing laboratory prior to the start of conditioning. If an attached cord blood unit segment is available, cord unit identity will be confirmed by either Class I or II HLA typing prior to the start of conditioning. Cord units will be thawed in Dextran/albumin per the methods of Rubinstein et al and Kurtzberg et al \(^\text{14} \text{ 20}\). Cord units will be thawed and administered sequentially to the patient. The start of the cord blood infusions will be 2-5 hours apart. Start times, but not stop times for the cord blood infusions will be documented.

B. Patient (recipient) Treatment:

1. All patients will sign an informed consent and register through the Dana Farber QACT.
2. Conditioning therapy with cyclophosphamide 1800mg/m\(^2\)/day IV Days -5,-4 (total dose 3600mg/m\(^2\)), fludarabine, 25mg/m\(^2\)/day IVx 3 days Days -6,-5,-4 (total dose 75mg/m\(^2\)), total body irradiation 1400 cGy in 7 fractions Days -3,-2,-1 and 0..
   Fludarabine doses will be based on actual weight. Cyclophosphamide doses will be based on actual body weight for those patients ≤ 125% ideal body weight. For those patients >125% ideal body weight, cyclophosphamide dose will be based on adjusted body weight. The adjusted body weight = ideal body weight + 0.4x (actual body weight – ideal body weight.)
3. GVHD prophylaxis with tacrolimus and mycophenolic mofetil (MMF). Tacrolimus (FK-506) will be given starting on Day -3 at a dose of 0.05 mg/kg orally bid. Tacrolimus may be given IV if the patient is not able to tolerate oral medication. Subsequent dosing will be based on clinical toxicity, GVHD, concurrent medications, medical conditions, drug levels, drug-drug interactions and blood levels with a target of 5-10ng/ml. Patients will be treated with oral tacrolimus when they are able to take oral medications. The oral dose of tacrolimus may be rounded to the nearest 0.5 mg. In the absence of GVHD and at the
discretion of the attending physician, tacrolimus will begin to be tapered by approximately Day +100 post transplant, with the goal of discontinuation of immunosuppression by 6-9 months post transplant.

MMF will be given at a dose of 15 mg/kg IV twice daily starting on Day –3 prior to transplant and continuing until Day +60 post transplant. The dose will be switched to oral MMF 15 mg/kg po twice daily (rounded to the nearest 250 mg dose) when the patient is able to take po. At the discretion of the attending physician and in the absence of acute GVHD, MMF will begin to be tapered on approximately Day +60 after transplant, with the goal of discontinuation of MMF by about Day +100 post transplant.

4. Parathyroid Hormone (PTH) will be given subcutaneously from Day +1 after transplant until day+29 after transplant, or when the ANC >2000 for 2 consecutive days, whichever comes first. PTH will be given as follows: 40 mcg on Day +1, 60 mcg on Day +2, 80 mcg on Day +3, and 100 mcg from Day +4 to Day +29 or until ANC >2000.

5. Post-transplant supportive care (antibiotics, transfusional support, etc.) (Section XIV)

6. It is estimated that the patient would be hospitalized for approximately 5-6 weeks. However, if a medical complication, for example infection or graft versus host disease, develops, the hospital stay could be prolonged. Patients may need to be readmitted to the hospital if a medical complication develops.

7. After hospitalization, patients will be followed closely in the BMT outpatient clinics. Patients will receive standard post transplant discharge teaching and guidelines to prevent infection.

C. Schema

<table>
<thead>
<tr>
<th>DAY</th>
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<tr>
<td>-6</td>
<td>Fludarabine 25 mg/m² IV</td>
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<td>Fludarabine 25mg/m² IV  Cyclophosphamide 1800 mg/m² IV</td>
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<td>Fludarabine 25 mg/m² IV  Cyclophosphamide 1800 mg/m² IV</td>
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<tr>
<td>-3</td>
<td>Total Body Radiation 200 cGy bid MMF/Tacrolimus</td>
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<tr>
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<td>Total Body Radiation 200 cGy x 1 ↓</td>
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<tr>
<td>0</td>
<td>Sequential cord blood infusions ↓</td>
</tr>
<tr>
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<td>Start PTH 40 mcg sc</td>
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VIII. Correlative Science Studies

Correlative science studies will be optional and designed to study the effect of PTH on immune reconstitution post cord blood transplantation. The recovery of naïve and memory T and B cell subsets, as well as NK cells, will be investigated. Peripheral blood will be obtained from consenting patients pre transplant and at approximately weeks 4 +/- 3 days, 100 days +/- 14 days, and months 12 +/- 14 days and 24 +/- 14 days. In addition, the discard from the thawed cord blood units will also be tested in consenting patients. Blood for correlative science studies will be coordinated to the patient’s other blood tests and clinic schedule.

Correlative science work will be performed in the laboratory of Dr. Vicki Boussiotis at Massachusetts General Hospital. Absolute lymphocyte counts, CD3+ T cells, CD19/CD20 B cells, NK (CD3-CD56+) cells, NK T cells (CD3+CD56+) and CD14+ monocytes will be assessed. The CD4/CD8 ratios will also be assessed, since previous studies have shown distinct kinetics in the recovery of CD4 and CD8 cells after BMT. T cell effector functions are mediated by memory (peripherally primed) cells. In addition, the influx of de novo-generated naïve cells is recognized as an important component of immune tolerance. Therefore, in addition to those naïve cells transferred with the UCB graft, de novo generated naïve cells may contribute to a reduced frequency of memory cells in UCB recipients. To assess the effects of UCB transplantation on the size of naïve and memory T cell pools we will use phenotypic markers that allow us to distinguish various functional differentiation stages, including naïve (T naïve), central memory (T cm), and effector memory (Tem) cells based on immunophenotype profiles previously established. We will put particular emphasis on the assessment of natural and adaptive T reg. Longitudinal analysis of the frequency of these populations will be conducted.
IX. Treatment Modalities

A. Fludarabine

1. Fludarabine is administered at a dosage of 25 mg/m² on Days –6,-5,-4 (total dose 75mg/m²). Fludarabine is dissolved in 100 ml (maximum concentration of 10 mg/ml) of standard IVPB fluids (i.e., dextrose 5% in water, normal saline) and administered as a bolus infusion over approximately 30 minutes, as per institutional guidelines. Fludarabine is commercially available.

2. Sedation and anti-nausea medications will be given per institutional guidelines.

3. Toxicity and Complications:
   a. The most common adverse events include myelosuppression, fever and chills, and nausea and vomiting.
   b. Other commonly reported events include malaise, fatigue, anorexia and weakness.
   c. Serious opportunistic infections have occurred in patients with CLL treated with fludarabine and are due to myelosuppression and prolonged impairment of cell mediated immunity.
   d. Neurotoxicity
      i. Peripheral neuropathy has been reported although would be unusual following a single course of therapy
      ii. CNS toxicity is rare following a single course of therapy (at a dose of 125mg/m²). Possible manifestations include delirium, seizures, coma, and visual disturbances due to optic neuritis.

B. Cyclophosphamide (Cytoxan)

1. Cyclophosphamide 1800 mg/m² q day x 2 days, Days –5,-4 CYCLOPHOSPHAMIDE will be given at least 2 hours after the start of the Fludarabine infusion.

2. Cyclophosphamide doses will be based on actual body weight for those patients ≤ 125% ideal body weight. For those patients >125% ideal body weight, cyclophosphamide dose will be based on adjusted body weight. The adjusted body weight =ideal body weight + 0.4x (actual body weight –ideal body weight.) Ideal body weight is defined per the following formula:
   Male= 50kg + (2.3x (inches taller than 60 inches))
3. Cyclophosphamide is commercially available and is administered intravenously. Cyclophosphamide is administered in 500-1000cc of Normal Saline and administered over 1-3 hours. Cyclophosphamide is an effective drug against many hematologic cancers and is also an immunosuppressant.

4. Sedation, intravenous fluids, mesna, and anti-nausea medications will be given per institutional guidelines. Mesna may also be administered depending on institutional practice. Mesna is used to reduce risk of hemorrhagic cystitis. The recommended dose is 15mg/kg IV bolus given 15 minutes before and 3, 6, and 9 hours after cyclophosphamide.

5. Toxicity and Complications:
   a. The most common adverse events include myelosuppression, alopecia, and nausea and vomiting.
   b. Hemorrhagic cystitis may occur and can be prevented with vigorous hydration and mesna.
   c. Cardiomyopathy may occur, especially when cyclophosphamide is given in higher doses.
   d. Other commonly reported events include malaise, fatigue, anorexia and weakness. Long-term side effects include infertility and increased risk of second cancers.

C. **Total Body Radiation**

Total Body Radiation will administered according to institutional practices, under the guidance of a radiation oncologist. TBI will be given for a total dose of 1400 c Gy in 7 fractions. TBI delivers effective anti cancer treatment, as well as suppression of the immune system. Toxicity and Complications:

   a. Common side effects include nausea, diarrhea, hair loss, parotid gland swelling, and infertility.
   b. Skin rashes may also occur.
   c. Suppression of the thyroid gland is common after TBI.
   d. The TBI will cause myelosuppression.
   e. Less common side effects include liver, kidney and lung damage, that may occur many months after treatment.
   f. Increased risk of cataracts

D. Tacrolimus (Tacrolimus, FK-506)

1. Tacrolimus is commercially available. Prograf capsules (Tacrolimus capsules) 0.5, 1 and 5 mg are stored at controlled room temperature, 15-30 degrees C). Tacrolimus is also available
intravenously and may be given IV if the patient cannot tolerate oral dosing. Tacrolimus is used to prevent serious graft versus host disease and graft rejection. Tacrolimus will be given at a dose of 0.05 mg/kg orally bid starting Day -3. The oral dose of tacrolimus may be rounded to the nearest 0.5 mg. Subsequent dosing will be based on toxicity, GVHD, concurrent medications, medical conditions, prior drug levels, drug-drug interactions and blood levels with a target of 5-10ng/ml. Levels will be checked approximately two to three times weekly during the initial hospitalization, then as clinically indicated.

The patient will be treated with oral dosing when the patients can reliably take oral medications.

In the absence of GVHD and at the discretion of the attending physician, tacrolimus will begin to be tapered on approximately Day +100 (+/- 2 weeks) post transplant, with the goal of discontinuation of Tacrolimus by 6-9 months post transplantation. The tapering schedule will be left to the treating physician’s discretion.

2. Toxicity and Complications:
The primary toxicities are reversible renal dysfunction (doubling of creatinine in 82%), hypertension requiring the use of antihypertensive medications (21%), and hyperglycemia (12%). In addition, hypomagnesemia, hypokalemia, and neurologic toxicity may occur. There is an increased risk of opportunistic infections and secondary malignancies.

E. Mycophenolate Mofetil

Mycophenolate Mofetil (MMF)

1. MMF is an immunosuppressant drug that is commercially available. It can be given either IV or po when the patient is able to tolerate po. The dose is 15mg/kg BID, with oral doses rounded to the nearest 250 mg. The first dose of MMF will be given on day -3. In the absence of GVHD and at the discretion of the attending physician, the dose will be tapered beginning on approximately day +60, with the goal of discontinuation of MMF on about Day +100 after transplant. The tapering schedule will be left to the treating physician’s discretion.

Side Effects May Include:

a. Depression of blood counts
b. Nausea
c. Diarrhea
d. Rarely, liver and kidney damage
F. **G-CSF (filgrastim, Neupogen)**

1. Patients will receive G-CSF 5mcg/kg/day SC or IV from day +5 post-transplant until engraftment (ANC >2000 x 2 days). The G-CSF dose may be rounded as follows: Patients < 65 kg, receive 300 mcg, patients 66-100 kg, receive 480 mcg, patients > 100 kg receive 600 mcg. The G-CSF is to accelerate engraftment, which is typically prolonged after cord blood transplant. Side effects may include:

   a. Bone pain described as mild to moderate
   b. Myalgia, fatigue, headache and insomnia
   c. Pain at the site of the injection
   d. Fever and nausea are uncommon
   e. Thrombocytopenia
   f. Hyperuricemia with gout
   g. Elevated liver function tests (transient)
   h. Splenic rupture (rare)

G. **Parathyroid Hormone**

1. Parathyroid hormone (teriparatide) is available commercially from Lilly and is administered sc daily from Days +1-+29 post transplant or until the absolute neutrophil count (ANC) >2000, whichever comes first. PTH will initially be given in the hospital. If the patient is discharged and still requiring PTH, PTH may be administered either in the outpatient clinic or at home via visiting nurse or the patient/family.

   The patient and/or family members will be trained in the technique of sc injections and the use of the multi dose pen. The dose will not be adjusted for weight. Dosing is as follows:

   Day +1: PTH 40 mcg
   Day +2: PTH 60 mcg
   Day +3: PTH 80 mcg
   Day +4 to Day+29 or until ANC >2000 PTH 100 mcg

   Calcium and ionized calcium levels will be followed at least three times weekly for patients taking PTH. Calcium levels will be drawn before the PTH dose. PTH will be held for a calcium level of >11.5 or an ionized calcium of >1.5. Routine treatment for hypercalcemia (fluids, Lasix) will be given to the patient at the treating physician’s discretion. PTH will be restarted when the calcium is< 10.5 and ionized calcium < 1.3. If a patient has >2 episodes of a calcium >11.5 or an ionized calcium >1.5, the PTH will be discontinued.
2. Toxicity and Complications:
   a. The most common adverse events include headache, nausea, hypercalcemia, joint aches, muscle aches, and frequent urination.
   b. Occasional dizziness, confusion, and orthostatic hypotension have been reported.
   c. Rats that have been given lifetime injections of PTH had an increased incidence of sarcoma. However, no increased incidence of cancer has been seen in either primate or human trials.
   d. It is recommended that patients do not routinely take Tums or other calcium supplements while taking PTH. Intravenous or oral calcium supplementation may be necessary during hospitalization based on calcium levels, and will be at the treating physician’s discretion.
   e. Fever may occur after PTH administration. Tylenol may be taken as needed.

H. Supportive Care

1. In order to minimize the chance of infection related to impaired immune reconstitution following the transplant, the following measures will be undertaken.
   a. Bacterial and fungal culture of all processed products.
   b. Monitoring for infection post-transplant including surveillance blood cultures and routine CMV antigenemia or hybrid capture testing (with preemptive ganciclovir or valganciclovir therapy in patients who develop a positive assay).
   c. Studies of immune reconstitution including approximately monthly quantitative immunoglobulin assays (with supplemental IVIG 400mg/kg for patients with an IgG of less than 400) for approximately the first six months after transplant.
   d. All patients will receive standard anti-infective prophylaxis including PCP prophylaxis with Bactrim or alternative from Day-8 to Day -1, and then from approximately Day +30 to either one year post transplant or until off all immunosuppressive therapy.
   e. Acyclovir 400 mg po tid (or alternative antiviral agent) until approximately one year post transplant.
   f. All patients will receive anti fungal prophylaxis with fluconazole or alternative antifungal agent for at least 100 days post transplant.
   g. Monitoring for EBV post transplant at least monthly for approximately the first six months post transplant using the EBV
Quantitative viral load assay.

- Monitoring for Human Herpes Virus 6 by peripheral blood PCR pretransplant, and approximately every two weeks until 8 weeks post transplant.
- A patient diary is not required for outpatient medicines on this protocol.

## I. Sequential Cord Blood Infusions

1. Umbilical cord blood from each HLA 4/6, 5/6, or 6/6 identical units will be thawed using a Dextran/Albumin wash. The cell count from both units will be >3.7 x 10^7 NC/kg pre-freeze. Post-thaw cell counts, sterility, viability, and flow cytometry for CD3, CD4, CD8, CD19, CD34, and CD56 assays will be performed, assuming there is enough excess cord blood material. Each cord blood unit will be thawed and administered separately. Potential toxicities of the stem cell infusion are infection and rarely allergic reaction.

## X. Evaluation

### A. Pre-transplant (all required tests must be performed within 30 days prior to registration or within 42 days prior to admission for transplantation)

1. **History.** A complete history with full details of the patient's previous treatment and response will be obtained. The complete history may be performed more than 30 days prior to registration.

2. **Clinical evaluation:**
   - A complete physical examination.
   - Chest and other radiographs as clinically indicated.
   - Marrow aspiration and biopsy for staging, cytogenetics, flow cytometry
   - EKG
   - Dental consult and evaluation of status of teeth and gums.
   - Baseline pulmonary function tests.
   - Echocardiogram or nuclear medicine test to assess ejection fraction.

3. **Laboratory data:**
   - ABO and Rh typing.
   - HLA typing of patient and available family members. The patient will be typed at HLA A, B, DR by high resolution
molecular typing and HLA antibodies (panel reactive antibodies) will also be tested. The HLA typing may be performed more than 30 days prior to registration.

c. Hepatitis B surface antigen, HCV, HSV, CMV, EBV. HIV and HTLV-I antibody determinations for patient as per institutional guidelines

d. PTH level, calcium level, ionized calcium level, phosphate level, 25-hydroxy Vitamin D level

e. CBC, reticulocyte count, comprehensive chemistry profile as per institutional guidelines.

f. Chimerism samples collected on patient and cord units prior to transplant. If there is no extra cord blood material available pre-transplant, chimerism studies may be obtained from the thawed cord blood units.

g. Flow Cytometry for CD3, CD4, CD8, CD19, CD34, CD56

h. Peripheral blood PCR for human herpes virus 6.

i. Optional research bloods (2 yellow top tubes) pre transplant and discard from thawed cord blood units to laboratory of Dr. Vicki Boussiotis at MGH.

B. Evaluation during conditioning and the first 100 days post-transplantation (see also section XIII):

a. CBC and chemistries per institutional guidelines.

b. Calcium level, ionized calcium level, and phosphate level approximately three times a week while on PTH

c. Weekly PTH level while on PTH

d. Marrow aspiration and biopsy with cytogenetics, flow cytometry, and chimerism on approximately day 100 +/- 14 days post-transplant.

e. Chest x-ray and pulmonary function tests as clinically indicated prior to Day +100. CXR and pulmonary function tests on Day +100 +/- 14 days post transplant.

f. Blood for tacrolimus levels as clinically indicated

g. Daily evaluation for acute GVHD during hospitalization, then approximately every other week until the Day +100 visit (Day 100 +/- 14 days post-transplant (see section XII)

h. Chimerism studies by short tandem repeats and/or flow cytometry at weeks 2 +/- 3 days, 4 +/- 3 days, 6 +/- 3 days, 8 +/- 3 days, and 10 +/- 3 days, then 100 days +/- 14 days, then at months 6 +/- 14 days, 12 +/- 14 days, and 24 +/- 14 days post transplant (peripheral blood). As possible, chimerism studies will include all cells, myeloid cells, and T cell subsets.

i. Lymphocyte recovery studies (by flow cytometry) to include CD3, CD4, CD8, CD19, CD34, and CD 56 at weeks 4 +/- 3 days, 8 +/- 3 days, then 100 days +/- 14 days, then months
6 +/- 14 days, 12 +/- 14 days, and 24 +/- 14 days.

j. Optional research bloods (2 yellow top tubes to laboratory of Dr. Vicki Boussiotis) at weeks 4 +/- 3 days, then 100 days +/- 14 days, then months 12 +/- 14 days and 24 +/- 14 days.

k. CMV, EBV studies, HHV 6 PCR and IgG levels as per section IXH above.

C. Evaluation following 100 days post-transplant

1. Marrow aspiration with cytogenetics, flow cytometry, and chimerism at 100 +/- 14 days, then months 6 +/- 14 days and 12 +/- 14 days.

2. Monthly evaluation or as clinically indicated for acute and chronic GVHD for the first six months post transplant.

3. Tumor response evaluation at 100 days +/- 14 days, 6 months +/- 14 days, 12 months +/- 14 days, and 24 months +/- 14 days. (See section XI).

4. Subjects will be followed longitudinally after completion of the study period for determination of disease-free and overall survival.

5. Long term follow up will be performed per institutional guidelines.

D. Subjects may be taken off study if

1. The subject wishes to be removed from the study.

2. The investigator feels this study protocol would not be in the subject's best interest.

3. The subject needs a medication that is not part of this study (e.g. chemotherapy for relapsed disease, second transplant) Subjects removed from the study will still be followed for survival and disease-free survival.

XI. Criteria for Anti-Tumor Response

Tumor assessments will be performed at baseline, 100 days, 6 months, 12 months (all +/- 14 days), then yearly or as clinically indicated and may include CT, gallium or PET scans, bone marrow aspirate and biopsy (if previously positive for tumor) and evaluations of other areas of known tumor involvement. For visits during which tumor evaluations are planned, the following definitions are to be used to assess the response.

A. Complete response:

There is no evidence of tumor after evaluation of all areas known to be previously involved, and no evidence of new tumor in any other location. For acute myelogenous leukemia and acute lymphoblastic leukemia, complete remission is defined as <5% blasts in the marrow with peripheral count recovery.
B. **Partial response:**

There has been more than a 50% regression in all measurable areas of previous tumor involvement, and no evidence of new tumor in any other location.

C. **No response / Progressive disease:**

Tumor regression less than a partial response or progression of disease in previous or new sites.

D. **Early death:**

Patient died prior to a scheduled tumor evaluation, and anti-tumor response cannot be assessed. Cause of death, such as infection, GVHD, organ toxicity shall be recorded.

XII. **Staging and Grading of Graft-vs-Host Disease**

A. **Acute GVHD usually occurs within the first 100 days following transplantation. The primary target organs of acute GVHD are skin, liver and the gastrointestinal tract. The staging and overall clinical grading severity of acute GVHD may be classified according to either the consensus or IBMTR scale.**

B. 1. **Organ Staging of GVHD**

<table>
<thead>
<tr>
<th>Stage</th>
<th>Skin</th>
<th>Liver</th>
<th>Gut</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Maculopapular rash &lt; 25% of body surface</td>
<td>Bilirubin 2-3 mg/dl</td>
<td>500-999ml diarrhea/day</td>
</tr>
<tr>
<td>2</td>
<td>Maculopapular rash, 25-50% of body surface</td>
<td>Bilirubin 3.1-6 mg/dl</td>
<td>1000-1499 ml diarrhea/day</td>
</tr>
<tr>
<td>3</td>
<td>Maculopapular rash, &gt;50% of body surface</td>
<td>Bilirubin 6.1-15 mg/dl</td>
<td>1500 or more ml diarrhea/day</td>
</tr>
<tr>
<td>4</td>
<td>Generalized erythroderma with bullae formation</td>
<td>Bilirubin &gt;15mg/dl</td>
<td>Severe abdominal pain with or without ileus</td>
</tr>
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</table>

1. **Overall Clinical Grading of Severity of Acute GVHD**
IBMTR GVHD Severity Index

<table>
<thead>
<tr>
<th>Index</th>
<th>Maximum Organ Stage</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>S=0, G=0, L=0</td>
</tr>
<tr>
<td>A</td>
<td>S=1, G=0, L=0</td>
</tr>
<tr>
<td>B</td>
<td>S, G, and/or L=2, or G and/or L=1</td>
</tr>
<tr>
<td>C</td>
<td>S, G, and/or L=3</td>
</tr>
<tr>
<td>D</td>
<td>S, G, and/or L=4</td>
</tr>
</tbody>
</table>

The IBMTR reviewed 2881 adults transplanted for leukemia between 1986-1992. They have proposed a new Severity Index grading scheme, which eliminates performance status as criteria.  

**B.** Chronic GVHD may occur in approximately 1/3 to 1/2 of patients with acute GVHD as the most important risk factor. Clinical manifestations are similar to the clinical sequelae of collagen vascular disease including sicca syndrome, sclerodermatous skin changes, pulmonary fibrosis, myositis / arthritis, and are typically reported as occurring greater than 100 days post-transplant.

XIII. **Supportive Care**

**A.** Access to vessels. All patients will have placement of an indwelling central venous catheter on or before admission.

**B.** Patients will receive hyperalimentation and other standard supportive care for bone marrow transplant patients according to institutional guidelines.

**C.** Transfusions: Transfusion practices will follow institutional guidelines. All blood products will be irradiated and leukoreduced.

**D.** Management of infections. Principles of infection prophylaxis and treatment will vary according to the spectrum of organisms and their antibiotic sensitivity and concurrent infection management practices and/or antibiotic clinical trial participation.

XIV. **Study Parameters**

<table>
<thead>
<tr>
<th></th>
<th>Pre Transplant</th>
<th>During PTH Treatment (also see Sections IX and XIII)</th>
<th>100 days +/- 14 days</th>
<th>6 months +/- 14 days</th>
<th>Months 12 and 24 +/- 14 days</th>
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<tr>
<td>History and Physical</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>CBC with</td>
<td>X</td>
<td></td>
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<td>X</td>
<td>X</td>
</tr>
<tr>
<td>Test</td>
<td>Pre Transplant</td>
<td>During PTH Treatment (also see Sections IX and XIII)</td>
<td>100 days +/- 14 days</td>
<td>6 months +/- 14 days</td>
<td>Months 12 and 24 +/- 14 days</td>
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<td>-----------------------------</td>
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<tr>
<td>Diff, reticulocyte count</td>
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<td>Chem Profile</td>
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<td>ABO, Rh and HLA typing</td>
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<tr>
<td>PTH level</td>
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<td>X (weekly)</td>
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<tr>
<td>25-hydroxy Vitamin D level</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>Calcium, albumin, ionized calcium, phosphate</td>
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<td>(three times a week)</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>HHV 6</td>
<td>X</td>
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<td></td>
<td></td>
<td>Every 2 weeks until 8 weeks</td>
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<td>CXR</td>
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<td>PFT's</td>
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<td>Echo or RVG</td>
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<tr>
<td>Bone Marrow asp/bx with chimerism studies/flow cytometry</td>
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<td>Tumor Staging</td>
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**Pre Transplant**

<table>
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<th>Study Protocol Details</th>
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<tr>
<td>Months 12 and 24 +/- 14 days</td>
<td>See Section X for additional chimerism assays, flow cytometry, CMV and EBV monitoring and research bloods. Specific GVHD studies will be performed as follows:</td>
</tr>
<tr>
<td>6 months +/- 14 days</td>
<td></td>
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<tr>
<td>100 days +/- 14 days</td>
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**Studies**

<table>
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<th>1 Year</th>
<th>2 Year</th>
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</thead>
<tbody>
<tr>
<td>X</td>
<td>As clinically indicated</td>
<td>As clinically indicated</td>
<td>As clinically indicated</td>
<td>As clinically indicated</td>
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</table>

Specific GVHD studies will be performed as follows:

<table>
<thead>
<tr>
<th>Days 0-28</th>
<th>Day 100</th>
<th>1 Year</th>
<th>2 Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tacrolimus level</td>
<td>Weekly</td>
<td>As clinically indicated</td>
<td></td>
</tr>
<tr>
<td>Skin Biopsy</td>
<td>As clinically indicated</td>
<td>As clinically indicated</td>
<td>As clinically indicated</td>
</tr>
<tr>
<td>Ophthalmology evaluation with Shirmer’s test</td>
<td>As clinically indicated</td>
<td>As clinically indicated</td>
<td></td>
</tr>
</tbody>
</table>

**XV. Data Management**

A. The data management team will consist of the BMT Program CRA / CRC.

Data will be entered into a computerized database using Access and Prophet systems. These data will include clinically relevant parameters such as regimen related toxicities, incidence of acute and chronic GVHD, regimen related mortality, progression free and overall survival. The database has limited access to protect patient confidentiality.

**XVI. Statistical Section**

**Primary Endpoint**

The primary objective is to evaluate the time to neutrophil engraftment (defined as ANC >500) among patients receiving parathyroid hormone following sequential unrelated cord blood transplantation. A median time of 23 days to neutrophil engraftment has been observed with the double cord blood approach after a myeloablative conditioning regimen. The goal of adding PTH is to improve the engraftment. PTH will be considered to be promising if the median time to neutrophil engraftment were reduced by 30% to 16 days.

The protocol design for the primary endpoint will be a single stage with an accrual goal of 40
patients. Allowing for the patients who may never achieve engraftment due to early transplant-related mortality or graft failure, we expect to have 35 patients with uncensored data on time to neutrophil engraftment. Assuming the time to neutrophil engraftment is exponentially distributed, the effective sample size of 35 patients will provide 80% power to detect a hazard ratio of 0.7 at a one-sided significance level of 10%.

**Stopping rule for grade III-IV acute GVHD**

Development of grade III-IV acute GVHD will be monitored closely during the study. Previous phase 1 data of single or sequential cord blood transplantation have reported grade III-IV acute GVHD in the 8%-20% range. Among the first 10 patients, if 3 or more patients experience grade III-IV acute GVHD, accrual will be temporarily put on hold and the protocol reassessed. Monitoring of these key safety endpoints will be conducted monthly and if the rates exceed the pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised.. The probability of stopping early is 0.62 if the true rate of grade III-IV acute GVHD were 30%. However, the early stopping rule is associated with probability of 0.07 if the true rate were only 10%.

In addition, the protocol will incorporate independent stopping rules for unacceptable transplant-related mortality and for excessive graft failure. Myeloablative transplant regimens are generally associated with early transplant-related mortality (TRM) in the 20-30% range. Among the first 10 patients, the protocol accrual will be stopped early if 4 or more patients were to die of TRM within the initial 100 days post-transplant. The decision rule is associated with 12% probability of stopping early if the overall rate of early TRM were truly 20%. The probability is 62% that at least 4 of the first 10 patients will die due to early TRM if the true rate were 40%.

The study design is based on an effective sample size that assumes about 10% of patients may never achieve engraftment, so the protocol accrual will stop as soon as graft failure occurs among more than 6 patients at any time during the accrual period. Primary graft failure is defined as lack of neutrophil engraftment by 42 days in patients surviving without relapse. If the true rate of graft failure were 20%, the probability is 71% for observing graft failure among 7 or more out of 40 patients. In contrast, only 10% probability is associated with the decision rule if the graft failure rate were truly 10%.

**Secondary Endpoints**

Other secondary endpoints include acute and chronic GVHD rate, platelet engraftment (plt >20K unsupported), pre-engraftment transfusion support, percent donor chimerism, 100-day transplant-related mortality, relapse rate, overall survival, and disease-free survival.

For acute grade II-IV GVHD, the 90% confidence interval will be no wider than 28% with a total accrual of 40 patients assuming none of them were to experience graft failure. Allowing for the patients who are expected to experience graft failure, the 90% confidence interval will be no wider than 30% in an effective sample size of 35 evaluable patients.
Number of RBC and platelet transfusions needed prior to engraftment as well as percent of donor chimerism will be calculated for each patient, and descriptive statistics (median, range) will be provided.

The cumulative incidence curves for chronic GVHD and relapse will be constructed reflecting time to relapse and chronic GVHD as competing risks.

The Kaplan-Meier method will be used to estimate time to platelet engraftment, overall survival, disease-free survival and 100-day transplant related mortality.

As the correlative studies for immune reconstitution will be optional, it is difficult to project the eventual number of patients who will submit samples. Therefore, the analysis of naïve and memory T and B cell subsets as well as NK cells will be limited primarily to basic summary measures at each of the scheduled time points. The course of immune recovery will be displayed graphically for individual patients if serial samples were available, but longitudinal modeling will not be feasible as the data are expected to be sparse.

XVII. Adverse Event Reporting

A. The relationship of each adverse experience to study medication or protocol will be recorded as one of the choices on the scale below:

- **NOT RELATED**  Unrelated to study medication therapy
- **POSSIBLE**  Unlikely relationship to study therapy, but cannot rule out relationship with certainty.
- **PROBABLE**  Relationship to study medication therapy is fairly certain.
- **DEFINITE**  Definite relationship to study medication
- **UNKNOWN**  Relationship to study medication is unknown.

B. The following events will be reported to the DFCI IRB, the NHLBI, and the FDA per FDA guidelines. This protocol is under IND 12767:

1. **Grade 2 or 3 Events**:
   a. Serious and Unexpected and possibly, probably, definitely associated with the intervention.
2. **Grade 4 Events**, if they are unexpected
3. **Grade 5 (Fatal) Event**:
   a. When subject is enrolled and actively participating in the trial or
   b. When event occurs within 30 days of last intervention
   c. If subject is in long term follow up, death is reported at continuing review.
C. Unexpected Adverse Experience:

Any adverse experience the specificity or severity of which is not consistent with the risk information provided to subjects in the consent form and the IRB.

1. All unexpected life threatening and fatal adverse events will be reported to the Institutional Review Board, the NHLBI, and the FDA within twenty-four hours of notification of the event. A written report will follow within ten working days. All life threatening and fatal expected toxicities (Grade III hematologic events excepted) will be reported according to DF/PCC and the FDA guidelines.

XVIII. References


