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

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I. Title: A Phase II Study of Parathyroid Hormone Following 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. Long-term disease free survival probabilities of up to 70-80% can be achieved, particularly in young patients with matched donors. Survival probabilities following unrelated donor transplantation range from 20-30% for high-risk patients to 60% for young patients with chronic myelogenous leukemia.

Given the size of most American families, only 30% of patients will have a matched sibling donor. 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. Although these registries have grown to include over four million volunteer donors, approximately 50% of patients are unable to find a suitably matched unrelated donor in time to proceed to transplant. It is particularly difficult for African Americans and other minorities to find matched unrelated donors.

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

The first related cord blood transplant was performed in 1988, in a child with Fanconi’s Anemia. The clinical experience with cord blood transplantation has recently been reviewed.

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 $3.7 \times 10^7$ NC/kg infused. The average nucleated cell dose of the cord blood units in many cord blood banks is $10 \times 10^8$ 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.

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 transplant. Sixty percent of patients developed Grades II-IV GVHD and 20% of patients developed Grades III-IV GVHD. Eighteen patients survive disease-free forty months after transplant. The median number of nucleated cells, prior to freezing and at thawing, was $2.1 \times 10^7$ NC/kg and $1.6 \times 10^7$ 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 a myeloablative non-total body irradiation (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. The median number of nucleated cells/kg infused was $1.7 \times 10^7$. 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 (TRM) 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 $1 \times 10^7$ NC/kg had a 75% probability of death; patients who received more than $3 \times 10^7$ 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 the cord blood transplant recipients received a cell dose of $> 2.0 \times 10^7$ 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 Center for International Blood and Marrow Transplant Research (CIBMTR) compared survival in adults with leukemia receiving unrelated bone marrow transplants versus unrelated cord blood transplants. Deaths related to infection were higher in the cord blood recipients, but acute GVHD was less likely in the cord blood group as 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.  

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. 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. Patients received 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 (ANC) of 500/µL 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 Minnesota group has also reported results following a double cord blood approach after a myeloablative conditioning regimen. 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 and TBI 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 reduced intensity 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 10⁷ 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 x 10⁷ 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 40% of patients. The one-year disease free survival was 67%.

Based on these encouraging results, a second reduced intensity double cord blood study using the same conditioning regimen with the GVHD prophylaxis of sirolimus and tacrolimus was undertaken. As of April 2007, 28 of the planned 32 patients have been enrolled. Preliminary results indicate a median day to neutrophil engraftment of 22 days (range 15-75 days). The median day to platelet...
engraftment was 46 days. Transplant related mortality was 17% at 100 days post transplant. Causes of death included sepsis, relapse, and post transplant lymphoproliferative disorder. With a median follow-up of 7 months, the overall survival at one year is 64%. Combining the patients on these two protocols yields 45 patients treated with the reduced intensity regimen of fludarabine/melphalan/thymoglobulin with a median day to neutrophil engraftment of 22 days.

### C. Experience with Ablative Conditioning Regimens

The optimal conditioning regimen for cord blood, and other stem cell transplants, is unknown. Historically, transplantation 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 myeloablative regimen chosen for this study, cyclophosphamide and TBI, is a standard ablative transplant conditioning regimen, in use for over 16 years. TBI 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.

Myeloablative regimens using total body radiation have been performed successfully in patients receiving cord blood transplants up to the age of 55. In the 2004 study, 21 patients over the age of 45 (median age 48 years) received total body radiation plus chemotherapy (either cyclophosphamide or cytosine arabinoside or both) followed by single cord blood transplant. Two patients died of transplant related toxicity prior to Day +100 and the two year disease free survival was 72%. In the most recent study, 11 patients with a median age of 51 received a myeloablative conditioning regimen of cyclophosphamide, cytosine arabinoside, and total body radiation followed by single cord blood transplantation. The 100 day transplant related mortality was 0. With a median follow up of 25 months, the probability of two year disease free survival is 73%. Therefore, the upper age limit for the myeloablative regimen is extended to age 50.

### 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 \(^{41}\). 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 \(^{31,32}\). 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/PTHr preceptor (PPR) \(^{42}\).

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 \(^{43}\).

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 \(^{44}\).

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 \(^{45}\). Human parathyroid hormone is clinically available and FDA approved for the treatment of osteoporosis in both men and women \(^{46}\).

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 \(^{46}\). The dose used in the women was 100 mcg PTH 1-84 daily \(^{47}\). 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\textsuperscript{48}.

A Phase I study of Parathyroid Hormone in Addition to G-CSF for Patients requiring Additional Stem Cell Mobilization has been completed at Massachusetts General Hospital, Dana Farber Cancer Institute, Beth Israel Deaconess Medical Center and MD Anderson Cancer Center\textsuperscript{49}. 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. Patients received 14 days of PTH, and G-CSF for the last four days.

Twenty patients were enrolled, and all completed the two-week treatment period. 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. Two of five patients who had failed two prior mobilization attempts had an adequate stem cell mobilization after receiving PTH.

\textbf{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. We add the reduced intensity regimen of fludarabine/melphalan/thymoglobulin used in over 45 patients with double cord blood transplants in Boston. Since the engraftment kinetics of these two regimens are similar, both regimens can be compared against historical controls not treated with parathyroid hormone. 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.

Patients over the age of 50 and/or have had a prior autologous stem cell transplant are not eligible for the myeloablative conditioning regimen. Patients who have had prior radiotherapy may not be eligible for the myeloablative conditioning regimen; these patients will need to be reviewed by radiation oncology. In other cases, the suitability of patients for either conditioning regimen 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 non-myeloablative regimens (such as aplastic anemia, untreated myelodysplasia) may be more appropriate for a myeloablative conditioning regimen. Those patients under age 50 who meet
eligibility criteria for the myeloablative approach but for whom the treating investigator prefers the reduced intensity regimen must have approval by the principal investigator prior to enrollment.

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/µL.

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 for myeloablative regimen of cyclophosphamide/total body radiation/fludarabine 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. Complete remission or CRp as defined by WHO criteria are acceptable.50

2. Age 18-50 years.
3. No prior autologous stem cell transplant.
4. ECOG Performance status of ≤2.
5. Patients must have two partially HLA-matched UCB units. Each unit must match at a minimum of 4 of 6 at HLA-A, -B, -DRB1 loci with the recipient and with each other. Unit selection is based on cryopreserved nucleated cell (NC) dose and HLA-A, -B, and –DRB1 matching by molecular techniques (intermediate resolution for HLA-A, -B and high resolution for –DRB1).
6. 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.
7. 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.
8. DLCO ≥50% predicted (corrected for hemoglobin).
9. LVEF > 50%
10. Calcium <10.5 mg/dl, phosphate > 1.6 mg/dl.
11. Non-pregnant and non-nursing.

B. Inclusion Criteria for Reduced Intensity Regimen of Fludarabine/Melphalan/Thymoglobulin

1. Disease-specific criteria
   a. Non-Hodgkin’s lymphoma, or Hodgkin’s lymphoma: in Complete Remission ≥2 (second complete remission, third complete remission, etc) or in partial remission.
   b. Multiple myeloma: relapsed.
   c. Chronic lymphocytic leukemia, Rai stage III or IV, or lymphocyte doubling time of 6 months, or stage I-II, having progressed after ≥ 2 chemotherapy regimens, in partial remission.
   d. Acute myelogenous or lymphoblastic leukemia in second or subsequent remission or in first remission with adverse cytogenetics or antecedent hematologic disorder. Complete remission or CRp as defined by WHO criteria are acceptable.
   e. Chronic myelogenous leukemia in accelerated or second stable phase, or imatinib resistant and not eligible for an ablative transplant.
   f. Myelodysplasia, previously treated or not eligible for ablative transplant.
2. Age 18-65 years.
3. ECOG performance status of 0, 1, or 2.
4. Patients must have two partially HLA-matched UCB units. Each unit must match at a minimum of 4 of 6 HLA-A, -B, -DRB1 loci with the recipient and with each other. Unit selection is based on cryopreserved nucleated (NC) dose and HLA-A, -B, and –DRB1 matching by molecular techniques (intermediate resolution for HLA-A, -B and high resolution for –DRB1).
5. Total combined nucleated cell dose from the 2 cord blood units > $3.7 \times 10^7$NC/kg (pre-freeze dose). Each single cord blood unit cell dose must be > $1.5 \times 10^7$NC/kg.
6. Lack of 6/6 or 5/6 HLA-matched related, 10/10 matched unrelated donor, or unrelated donor not available within the time frame necessary to perform a potentially curative stem cell transplant.
7. DLCO ≥50% predicted corrected for hemoglobin.
8. LVEF > 45%.
9. Calcium < 10.5 mg/dl, phosphate > 1.6 mg/dl.

C. Exclusion criteria for eligibility for both regimens include:

1. Cardiac disease: symptomatic congestive heart failure, active angina pectoris, or uncontrolled hypertension.
2. Pulmonary disease: severe chronic obstructive lung disease, or symptomatic restrictive lung disease, or corrected DLCO of < 50% 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 seropositive.
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. Patients must have two partially HLA-matched UCB units. Each unit must match as a minimum of 4 of 6 HLA-A, -B, -DRB1 loci with the recipient. This may include 0-2 antigen mismatches at each A or B (at molecular intermediate resolution) or -DRB1 (at the allele level) loci. Each unit must be a 4-6 HLA-A, -B, and –DRB1 match to each other, not necessarily at the same loci as with the recipient. All typing will be done using molecular typing. Though molecular typing will be available, a match is defined at intermediate resolution for HLA-A and –B and at high resolution for –DRB1. HLA C and DQ will be tested when possible but will not be used in the match strategy.

2. All cord blood units will have confirmatory HLA typing performed prior to transplant. The confirmatory HLA typing may be performed at an accredited laboratory of the cord blood bank or at the participating transplant center.

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 CD34+ cell dose preferable).

2. HLA type (closer A, B DR match; DR match takes preference).

3. Age of unit (newer unit preferable).

VI. Evaluation, Counseling and Consent of Patient

Patients are referred for consideration of stem cell transplantation. Patients are completely evaluated per institutional guidelines, and 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 participating transplant center’s Institutional Review Board.

VII. Treatment Plan

A. Identification and Preparation of the Cord Blood Unit

Cord blood units will be identified through existing cord blood registries such as the American Red Cross, National Marrow Donor Program, New York Blood Center, etc. 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. Cord units will be thawed and washed and administered sequentially to the patient. The start of the cord blood infusions, when possible, 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 EMMES Corporation.

2. Myeloablative conditioning regimen: Conditioning therapy with cyclophosphamide 1800mg/m2/day IV Days –5,-4 (total dose 3600mg/m²), fludarabine, 25mg/m²/day IVx 3 days Days –6,-5,-4 (total dose 75mg/m²), 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.)

Reduced Intensity Conditioning Regimen: Conditioning therapy with fludarabine 30 mg/m²/day x 6 days, Days –8,-7,-6,-5,-4,-3 (total dose 180mg/m²), melphalan 100mg/m²/day x 1 day, Day –2, and rabbit antithymocyte globulin 1.5 mg/kg/day x 4 days, Days –7,-5,-3,-1 (total dose 6.0 mg/kg). Chemotherapy doses will be based on actual body weight.
3. GVHD prophylaxis with tacrolimus and mycophenolate mofetil (MMF). Doses will be based on actual body weight.

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 or equivalent level if using a different method. 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 oral medications. 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/µL 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/µL.

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

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 clinic. Patients will receive standard post transplant discharge teaching and guidelines to prevent infection.
### C. Schema:

Myeloablative Treatment Regimen

<table>
<thead>
<tr>
<th>Day</th>
<th>Conditioning Regimen</th>
<th>PTH and G-CSF</th>
<th>Immunosuppression</th>
</tr>
</thead>
<tbody>
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<td>PTH 100 mcg SQ daily until ANC &gt;2000 x 2 days or until Day +29, whichever comes first</td>
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<td>thru</td>
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<td>G-CSF 5 mcg/kg/day SQ/IV until ANC &gt;2000 µL x 2 days</td>
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<td>+28</td>
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<td>Last day of PTH, if still being given</td>
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<td>Continue G-CSF until ANC &gt;2000 µL x 2 days</td>
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<tr>
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## Schema: Reduced Intensity Regimen

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<td>PTH 40 mcg SQ</td>
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<td>• Last day of PTH, if still being given</td>
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<td>• Continue G-CSF until ANC &gt;2000/µL x 2 days</td>
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<tr>
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<td>Begin taper MMF</td>
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<tr>
<td>+100</td>
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<td>Begin taper Tacrolimus</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. These studies will only be performed for those patients treated in Boston. 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 rations 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/m^2 on Days –6,-5,-4 (total dose 75mg/m^2) for the myeloablative regimen. Fludarabine is administered at a dosage of 30mg/m^2 on Days –8,-7,-6,-5,-4,-3 (total dose 180 mg/m^2) in the reduced intensity regimen. Fludarabine is dosed on actual body weight. 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 lymphoid malignancy 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. Possible manifestations include delirium, seizures, coma, and visual disturbances due to optic neuritis.

B. Cyclophosphamide (Cytoxan)

1. 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= $50kg + (2.3 \times (\text{inches taller than } 60 \text{ inches}))$

   Female= $45.5 \text{ kg} + (2.3 \times (\text{inches taller than } 60 \text{ inches}))$

   Rounded to 1 decimal place

2. 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.

3. 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.

4. 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 Irradiation

Total Body Irradiation will administered according to institutional practices, under the guidance of a radiation oncologist. TBI will be given for a total dose of 1400 cGy in 7 fractions, in the myeloablative regimen. 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. Melphalan

1. Melphalan is commercially available and is administered intravenously. Melphalan is dosed on actual body weight. The dose is 100mg/m² IV given as one dose on day -2 (total dose 100mg/m²). Melphalan must be given immediately after reconstitution and infused over approximately 30-60 minutes, as per institutional guidelines. Sedation and anti nausea medications will be given per institutional guidelines.

2. Toxicity and Complications:

The most common side effects are myelosuppression, mucositis, nausea, and alopecia. Melphalan is a vesicant and can cause skin damage if there is skin infiltration. Other side effects include pulmonary fibrosis and liver damage.

E. Rabbit Antithymocyte globulin (Thymoglobulin; Genzyme)

1. Rabbit antithymocyte globulin (Thymoglobulin) is commercially available and is given intravenously at a dose of 1.5mg/kg/day on days -7,-5,-3,-1 (total dose 6.0mg/kg). Thymoglobulin is dosed on actual body weight. Thymoglobulin is diluted in D5W or NS and filtered with a 0.22-micron filter. The medication should be given over approximately 6 hours on the first day and over approximately 4 hours on subsequent days, as per institutional guidelines. Suggested premedications are Benadryl 50mg po or IV, Tylenol 650 mg po, and Solu-Medrol
40 mg IV. An anaphylaxis kit should be at the bedside.

2. Toxicity and Complications:
   Thymoglobulin can cause allergic reactions and serum sickness, including fever, rigors, muscle aches, and shortness of breath. The serum sickness may occur even after the last dose, and can last several days. Premedications can help to avoid serious side effects. The incidence of serum sickness and anaphylaxis is less than with equine antithymocyte globulin. Other side effects include immune suppression, headache, abdominal pain, nausea, diarrhea, dizziness, secondary lymphomas, peripheral edema, and renal failure.

F. 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 at a dose of 0.02mg/kg over 24 hours continuous infusion, 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 for both the myeloablative and reduced intensity regimens. Tacrolimus dosing is based on actual body weight. 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, hyperkalemia, and neurologic toxicity may occur. There is an increased risk of opportunistic infections and secondary malignancies.
G. 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 oral medication. The dose is 15mg/kg BID, with oral doses rounded to the nearest 250 mg. for both the myeloablative and reduced intensity regimens. MMF dosing will be based on actual body weight. 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. Liver damage (rare)
e. Kidney damage (rare)
f. Damage to unborn baby and limited effectiveness of birth control

H. G-CSF (filgrastim, Neupogen)

1. Patients will receive G-CSF 5mcg/kg/day SQ or IV from day +5 post-transplant until engraftment (ANC >2000/µL x 2 consecutive days), for both the myeloablative and reduced intensity regimens. G-CSF dosing is based on actual body weight. 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. For a decrease in neutrophil counts after engraftment, G-CSF may be reinstituted at the investigator’s discretion. 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)
I.  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 ANC >2000/µL, whichever comes first, for both the myeloablative and reduced intensity regimens.

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 SQ 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/µL: 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 at the last dose used when the calcium is < 10.5 mg/dL and ionized calcium < 1.3 mmol/L. If a patient has >2 episodes of a calcium > 11.5 mg/dL or an ionized calcium >1.5 mmol/L, the PTH will be discontinued.

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.
J. 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 or CMV testing per institutional guidelines (with preemptive ganciclovir or valganciclovir therapy in patients who develop a positive assay, as per institutional guidelines). CMV testing is recommended weekly through at least Day +100 after transplant.
   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 (per institutional guidelines) 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 per institutional guidelines (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 every other week for approximately the first six months post transplant using the EBV quantitative viral load assay. The risk of EBV lymphoproliferative disorder is 10% with the reduced intensity regimen and empiric Rituxan should be considered for rising EBV titers. Guidelines for management of elevated EBV titers are included in Appendix A.
   h. Monitoring for Human Herpes Virus 6 by peripheral blood PCR pretransplant and approximately every two weeks until 8 weeks post transplant.
   i. A patient diary is not required for outpatient medicines on this protocol.

K. Sequential Cord Blood Infusions

1. Umbilical cord blood from each HLA 4/6, 5/6, or 6/6 identical unit will be thawed using a Dextran/Albumin wash\(^{14}\).\(^{20}\). The cell count from both units will be $>3.7 \times 10^7$ 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, when
possible, approximately 2-5 hours should elapse between the start of the
cord blood infusions. When possible, the cord unit with the highest
nucleated cell dose pre freeze will be infused first. 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. A complete physical examination.
   b. Chest and other radiographs as clinically indicated.
   c. Marrow aspiration and biopsy for staging, cytogenetics, flow
cytometry
   d. EKG
   e. Dental consult and evaluation of status of teeth and gums (may be
      performed within 6 months prior to transplant).
   f. Baseline pulmonary function tests.
   g. Echocardiogram or nuclear medicine test to assess ejection fraction.

3. Laboratory data:
   a. ABO and Rh typing.
   b. HLA typing of patient and available family members. 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 with differential, 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, 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
B. Evaluation during conditioning and the first 100 days post-transplantation (see also section XIV):

a. CBC with differential 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 PCR methods and/or flow cytometry at weeks 2 +/- 3 days, 4 +/- 3 days, 8 +/- 3 days, and 100 days +/- 14 days, post transplant (peripheral blood). Chimerism studies must be performed on the total cells. If possible, chimerism studies will also be performed on myeloid cell and T cell fractions.
i. Lymphocyte recovery studies (by flow cytometry) to include CD3, CD4, CD8, CD19, and CD 56 at weeks 4 +/- 3 days, 8 +/- 3 days, and 100 days +/- 14 days
j. Optional research bloods (2 yellow top tubes to laboratory of Dr. Vicki Boussiotis) at weeks 4 +/- 3 days and 100 days +/- 14 days (for Boston patients only).
k. CMV, EBV studies, HHV 6 PCR and IgG levels as per section IX.J above.
l. Tumor response evaluation at 100 days +/- 14 days.
C. Evaluation following 100 days post-transplant

1. History and physical exam at months 6 +/- 14 days, 12 +/- 14 days, and 24 +/- 14 days.
2. CBC with differential and comprehensive chemistry profile at months 6 +/- 14 days, 12 +/- 14 days, and 24 +/- 14 days.
3. Marrow aspiration with cytogenetics, flow cytometry, and chimerism at months 6 +/- 14 days and 12 +/- 14 days.
4. Monthly evaluation or as clinically indicated for acute and chronic GVHD for the first six months post transplant.
5. Ophthalmology Evaluation with Shrimer’s Test, as clinically indicated at Day 100 +/- 14 days and month 12 +/- 14 days.
6. Tumor response evaluation at 6 months +/- 14 days, 12 months +/- 14 days, and 24 months +/- 14 days.
   (See section XI).
7. Peripheral Blood Chimerism studies at 6, 12, and 24 months +/- 14 days per section Bh.
8. Lymphocyte recovery studies (by flow cytometry) to include CD3, CD4, CD8, CD19, and CD56 at 6 months +/- 14 days, 12 months +/- 14 days, and 24 months +/- 14 days.
9. Optional research bloods (2 yellow top tubes to laboratory of Dr. Vicki Boussiotis) at months 12 +/- 14 days and 24 +/- 14 days (for Boston patients only).
10. Subjects will be followed longitudinally after completion of the study period for determination of disease-free and overall survival.
11. 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 CIBMTR scale.

B.

1. **Organ Staging of GVHD**

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<th>Stage</th>
<th>Skin</th>
<th>Liver</th>
<th>Gut</th>
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<tr>
<td>1</td>
<td>Maculopapular rash, &lt;25% of body surface</td>
<td>Bilirubin 2-3 mg/dl</td>
<td>500-999 ml diarrhea/day</td>
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<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>
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<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>
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<tr>
<td>4</td>
<td>Generalized erythroderma with bullae formation</td>
<td>Bilirubin &gt;15 mg/dl</td>
<td>Severe abdominal pain with or without ileus</td>
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</table>
1. Overall Clinical Grading of Severity of Acute GVHD

CIBMTR GVHD Severity Index

<table>
<thead>
<tr>
<th>Index</th>
<th>Maximum Organ Stage</th>
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<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

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<th>100</th>
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</tbody>
</table>

**Note:**
- <sup>1</sup> Mandatory every 4 months.
- <sup>2</sup> Mandatory every 6 months.
- <sup>3</sup> Mandatory every 12 months.
- <sup>4</sup> Optional every 4 months.
- <sup>5</sup> Optional every 12 months.
<table>
<thead>
<tr>
<th>Cytometry, and Chimerism</th>
<th>Tumor Staging</th>
<th>Research Bloods</th>
<th>Chimerism Studies</th>
<th>Flow Cytometry</th>
<th>Quantitative Immunoglobulin Assays</th>
<th>Acute and Chronic GVHD Evaluation, including biopsies as clinically indicated</th>
<th>Tacrolimus Levels</th>
<th>Ophthalmology Evaluation with Shimer's Test, as clinically indicated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X(\times)</td>
<td>X(\times)</td>
<td>X(\times)</td>
<td>X(\times)</td>
</tr>
</tbody>
</table>

1. Frequency of CBC with differential and chemistries in the first 100 days post-transplant is per institutional guidelines.
2. Dental consult and evaluation may be performed within 6 months prior to transplant.
3. EBV monitoring should be performed every other week for the first 6 months post-transplant.
4. HLA typing may be performed more than 30 days prior to registration.
5. Calcium level, ionized calcium level, and phosphate level to be performed three times a week while receiving PTH.
6. Optional research bloods (2 yellow top tubes to laboratory of Dr. Vicki Boussiotis) for Boston patients only.
7. Chimerism is to be performed by PCR methods and/or flow cytometry. Chimerism studies must be performed on the total cells. If possible, chimerism studies should include myeloid cell and T cell fractions.
8. Flow cytometry to include CD3, CD4, CD8, CD19, and CD56.
9. Daily evaluations for acute GVHD during hospitalization and approximately every other week post-discharge until Day 100 post-transplant. Acute and chronic GVHD evaluations monthly, or as clinically indicated, from Day 100 until six months post-transplant.
10. Tacrolimus levels should be checked two to three times weekly during the initial hospitalization, then as clinically indicated.
XV. Data Management

A. The data management team will consist of the BMT Program CRA / CRC.
   Centralized data for the study will be managed by the EMMES Corporation.
   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.

B. Enrollment Procedures

Patients will be registered using the SCCT Electronic Data Capture System
   (AdvantageEDC SM).

1. Prior to initiation of conditioning regimen, but no more than 14 days prior to initiation
   of conditioning regimen, an authorized user at the transplant center enters the
   patient demographics and Segment A of the Enrollment Form in AdvantageEDC.
   The eligibility screening (Segment A) includes a question confirming that the patient
   (or legal guardian) signed the informed consent.

2. If the patient is eligible, a patient identification number is generated.

3. A visit schedule based on treatment start date is displayed for printing and is
   referred to as ‘Segment A Follow-up.’

XVI. Statistical Section

Primary Endpoint

The primary objective is to evaluate the time to neutrophil engraftment (defined as ANC
>500/µL) among patients receiving parathyroid hormone following sequential unrelated cord
blood transplantation. The primary analysis will be based on the cumulative incidence of
neutrophil engraftment. A median time of 23 days to neutrophil engraftment has been
observed with the double cord blood approach after a myeloablative conditioning regimen 32.
A median time of 22 days to neutrophil engraftment has been observed with the double cord
blood approach after a reduced intensity conditioning regimen 33,34.

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. This study will be monitored by a DSMB from the NHLBI. 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%.

**Stopping rule for transplant-related mortality**

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. Reduced intensity double cord blood transplantation has been associated with transplant related mortality in the 15-20% 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%.

**Stopping rule for graft failure**

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 4 or more patients among the first 10 evaluable patients. 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 40%, the probability is 83% for observing graft failure among 4 or more out among the first 10 evaluable patients. In contrast, only 7% 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. ADVERSE EVENT DEFINITIONS

Adverse Event - Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure regardless of whether it is considered related to the medical treatment or procedure (attribution of definite, probable, possible, unlikely, or unrelated).

Life-Threatening Adverse Event - Any adverse event that places the participant, in view of the investigator, at immediate risk of death from the reaction.

Serious Adverse Event (SAE) - Any adverse event that results in any of the following outcomes:

- death,
- a life threatening adverse event,
- in-patient hospitalization or prolongation of existing hospitalization,
- a persistent or significant disability/incapacity,
- a congenital anomaly/birth defect, or
- any other medical event, in appropriate medical judgment, may require medical or surgical intervention to prevent one of the outcomes listed above.

Unexpected Adverse Event - For SCCT studies involving hematopoietic stem cell transplantation: any adverse event, the specificity or severity of which is NOT listed in the study protocol, product inserts or informed consent document.

Attribution - The determination of whether an adverse event is related to a drug/device/treatment. Attribution categories:
**Definite** - The adverse event is *clearly related* to the study drug/device/procedure/treatment(s).

**Probable** - The adverse event is *likely related* to the study drug/device/procedure/treatment.

*For SCCT studies involving hematopoietic stem cell transplantation: the adverse event is not likely to be caused by the subject’s underlying medical condition or other concomitant therapy, and the nature of the adverse event or the temporal relationship between the onset of the adverse event and study drug/device/treatment administration lead the investigator to believe that there is a reasonable chance of causal relationship.*

**Possible** - The adverse event *may be related* to the study drug/device/procedure/treatment(s).

For SCCT studies involving hematopoietic stem cell transplantation: the adverse event could be attributed to the subject’s underlying medical condition or other concomitant therapy, but the nature of the adverse event or the temporal relationship between the onset of the adverse event and study drug/device/treatment administration lead the investigator to believe that there could be a causal relationship.

**Unlikely** - The adverse event is *doubtfully related* to the study drug/device procedure/treatment(s).

**Unrelated** - The adverse event is *clearly NOT related* to the study drug/device/procedure/ treatment(s).

For SCCT studies involving hematopoietic stem cell transplantation: the adverse event is most plausibly explained by the subject’s underlying medical condition or other concomitant therapy, or the adverse event has no plausible biological relationship to study drug/device /treatment.

**Common Terminology Criteria Adverse Events (CTCAE)** – a descriptive terminology developed by the National Cancer Institute (NCI) for use in reporting adverse events. The CTCAE includes a grading (severity) scale for each adverse event term. A copy of the current CTCAE guidelines (Version 3.0) is located at [http://ctep.cancer.gov/reporting/](http://ctep.cancer.gov/reporting/).

**Grade** – Severity of the adverse event. Grades were developed using the following guidelines:

- **Grade 0** – No adverse event or within normal limits
  1 – Mild adverse event
  2 – Moderate adverse event
  3 – Severe adverse event
  4 – Life-threatening or disabling adverse event
  5 – Fatal adverse event
Abnormal laboratory values not included in the CTCAE guidelines will be defined per protocol.

B. ADVERSE EVENT REPORTING

Adverse events should be reported using CTCAE terminology and severity scales for all SCCT studies involving hematopoietic stem cell transplantation. Information reported for the adverse event must include: Name of adverse event, date of first onset, peak severity, relationship to study drug/device/treatment, resolution date, actions taken with respect to administration of study drug/device/treatment, and other treatment for the adverse event. Table 1.2.1 describes reporting time frames. Adverse events will be reported as long as specified in the protocol.

Any adverse event that is sent to the local IRB will be sent at the same time to the DCC. The DCC will ensure that the report contains sufficient information, will code it using MEDRA, and will review and distribute as described below.

C. SCCT MONITORING OF ADVERSE EVENTS

Table 1.2.1 summarizes the reporting requirements for expected and unexpected adverse events. The section below describes in more detail the reporting requirements.

**Unexpected Grades 3-5 Adverse Events**

- All unexpected Grades 3-5 adverse events will be reviewed by the Medical Monitor at the DCC, within 2 business days of receiving the adverse event form (or MedWatch form) from the clinical center.
- If the Medical Monitor requires additional information to make his/her assessment, the clinical centers will have 2 business days to respond to the request for additional information.
- The DCC is responsible for notifying the NHLBI Project Officer, or designated NHLBI medical monitor, immediately of all unexpected Grades 3-5 adverse events, regardless of attribution, and of any concerns regarding the frequency or type of adverse event(s) on a study or study treatment arm.
- The attribution, as assessed by the clinical center and the DCC medical monitor, will be provided to the NHLBI Project Officer or designated NHLBI medical monitor, within 2 business days but no later than 7 days of receiving the report.
- All unexpected Grades 3- 5 adverse events will also be sent to the DSMB.

The NHLBI Project Officer (or designee) is responsible for reviewing the adverse event materials to determine if the materials are complete. If there are any concerns regarding the type or frequency of the event, the NHLBI Project Officer will request that the DSMB Executive Secretary notify the DSMB. The DSMB will review the adverse event materials, determine if the information is complete, determine if additional DSMB review is required and make recommendations to the NHLBI concerning continuation of the study.
The DCC will prepare quarterly summary reports of all unexpected Grades 3-5 adverse events and annual summary reports of all unexpected Grades 1-2 for the NHLBI Project Officer (or designee) and DSMB. Quarterly reports will be made available on a secure website and the NHLBI Project Officer (or designee) and DSMB will be notified by e-mail when the materials are posted. The summary reports will include a minimum of:

- description of the adverse event,
- whether it is expected or unexpected,
- the degree of attribution,
- a clinical summary and conclusion.

**Expected Adverse Events**

- All expected Grade 5 adverse events will be reviewed by the Medical Monitor at the DCC, within 1 business day of receiving the adverse event form (or MedWatch form) from the clinical center.
- If the Medical Monitor requires additional information to make his/her assessment, the clinical centers will have 2 business days to respond to the request for additional information.
- The DCC is responsible for notifying the NHLBI Project Officer, or designated NHLBI medical monitor, immediately of all expected Grades 5 adverse events, regardless of attribution, and of any concerns regarding the frequency or type of adverse event(s) on a study or study treatment arm.
- The attribution, as assessed by the clinical center and the DCC medical monitor, will be provided to the NHLBI Project Officer or designated NHLBI medical monitor, within 2 business days, but no later than 7 days of receiving the report.
- All expected Grades 5 adverse events will also be sent to the DSMB.

The DCC will prepare quarterly summary reports of all expected Grades 3-5 adverse events and annual summary reports of all expected Grades 1-2 for the NHLBI Project Officer (or designee) and DSMB. The reports will be made available on a secure website and the NHLBI Project Officer (or designee) and DSMB will be notified by e-mail when the materials are posted. The summary reports will include a minimum of:

- description of the adverse event,
- whether it is expected or unexpected,
- the degree of attribution,
- a clinical summary and conclusion.

Any concern regarding the type or frequency of a Grade 3-5 expected adverse event will be reported to the NHLBI Project Officer who will determine if referral to the DSMB is warranted. If required, data materials will be provided by the DCC. The DSMB Executive Secretary will arrange for review by the DSMB Chair. The Chair will determine if additional DSMB review is required and make recommendations to the NHLBI concerning continuation of the study.
D. FDA IND/IDE Reporting

The sponsor of the IND/IDE is responsible for reporting to the FDA on at least an annual basis and for reporting expedited safety reports (21 CFR 312.32). Annual reports are due within 60 days of the IND/IDE submission anniversary. If a study is under an FDA IND or IDE, all unexpected adverse events are reported to the FDA by telephone or fax as defined in Table 1.2.1 below.
<table>
<thead>
<tr>
<th>Relationship</th>
<th>Grade</th>
<th>Attribution</th>
<th>Clinical Center Reporting Requirements to the DCC</th>
<th>DCC Reporting to NHLBI</th>
<th>FDA Reporting</th>
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<tbody>
<tr>
<td>Unexpected</td>
<td>1-2</td>
<td>Unrelated/ Unlikely Possible/ Probable/ Definite</td>
<td>Summarized annually</td>
<td>Summarized annually</td>
<td>Summarized annually</td>
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<tr>
<td></td>
<td>3-5</td>
<td>Unrelated/ Unlikely Possible/ Probable/ Definite</td>
<td>Report to the DCC within 1 business day.</td>
<td>Grades 3-5 within 2 calendar days but no later than 7 days</td>
<td>Grades 4-5 within 7 calendar days by telephone or fax. Grades 3-5 written report within 15 calendar days</td>
</tr>
<tr>
<td>Expected</td>
<td>1-2</td>
<td>Unrelated/ Unlikely Possible/ Probable/ Definite</td>
<td>Report to the DCC quarterly. Reportable events in this category will be defined on a study specific basis and captured on case report forms.</td>
<td>Summarized annually</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3-4</td>
<td>Unrelated/ Unlikely Possible/ Probable/ Definite</td>
<td>Summarized quarterly</td>
<td></td>
<td>Summarized annually</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>Any</td>
<td>Report to the DCC within 1 business day.</td>
<td>Within 2 calendar days but no later than 7 days</td>
<td></td>
</tr>
</tbody>
</table>

**PLEASE NOTE:** Any adverse event that is sent to the local IRB will be sent at the same time to the DCC. Grade 5 means a fatal event has occurred.
Appendix A

Epstein-Barr Virus (EBV) surveillance using a real time quantitative EBV DNA PCR plasma-based assay shall be performed every other week until 6 months post transplant, and then as clinically indicated. Patients who develop EBV DNA levels of > 1000 copies/mL on any tests will receive 375 mg/m² of rituximab. Those patients that continue to have levels above 1000 copies/mL on subsequent testing will receive three additional weekly infusions of 375 mg/m² of rituximab. Patients who develop a fever of unknown origin to > 39°C, lymphadenopathy, or hepatosplenomegaly, should undergo CT scanning of the chest and abdomen to rule out or stage EBV post-transplant lymphoproliferative disorder (PTLD). Tissue diagnosis that includes EBER and LMP-1 immunocytochemistry should be attempted. Other diagnostic or staging studies will be performed as clinically indicated. The principal investigator should be consulted for advice regarding treatment of suspected EBV PTLD.
XVIII. References


Marrow Transplant 2007; 13: 337a.


50 Cheson BD, Bennett JM, Kopecky KJ, et al. Revised Recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria,

