A Phase II Study of Reduced Intensity Double Umbilical Cord Blood Transplantation Using Fludarabine, Melphalan, and Low Dose Total Body Radiation

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Title: A Phase II Study of Reduced Intensity, Double Umbilical Cord Blood Transplantation Using Fludarabine, Melphalan, and Low Dose Total Body Radiation.

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Agent(s).
Fludarabine—Commerical Supply—APP and Bioniche Pharma
Melphalan—Commercial Supply—Novation
Total Body Radiation
Tacrolimus—Commercial Supply—Astellas Pharma
Sirolimus—Commercial Supply—Wyeth Pharmaceuticals
Granulocyte Colony Stimulating Factor (G-CSF)—Commercial Supply Neupogen—Amgen
Or Granix- Commercial Supply

Study Synopsis

A Phase II Study of Reduced Intensity, Double Umbilical Cord Blood Transplantation Using Fludarabine, Melphalan, and low dose Total Body Radiation.

Primary Objective: The primary objective of this Phase II trial is to determine the one year significant viral infection rate (viral infections requiring medical intervention) after double umbilical cord blood transplant using a novel conditioning regimen of fludarabine/melphalan/low dose total body radiation.

Secondary Objectives: Secondary objectives of this trial include the time to engraftment of neutrophils and platelets, primary graft failure, the rates of acute and chronic GVHD, relapse, 100-day treatment related mortality, post transplant lymphoma, measures of immune reconstitution, thrombopoietin levels, and relapse-free and overall survival at 1 and 2 years from transplantation.

Study Design: This is a Phase II study designed to test the new conditioning regimen. One year significant viral infection rate will be compared to our historical control population, which has a one-year significant viral infection rate of 53%.

Accrual Objective: The sample size is a maximum of 31 patients.

Accrual Period: The estimated accrual period is two and a half years.

Eligibility Criteria: Patients ages 18-65 years old with a diagnosis of hematological malignancy and with two partially HLA-matched unrelated cord blood units would be eligible. Patients with sibling donors and readily available 8/8 HLA-matched unrelated donors would not be eligible. Patients will have adequate measures of organ function prior to transplantation. Cord blood units must be HLA allele level matched at 4 of 6 HLA-A, B and DRB1 loci with each other and with the recipient. Two units must be available such that the total combined nucleated cell dose $\geq 3.7 \times 10^7$ nucleated cells/kg of recipient weight. Each single cord blood unit cell dose must be $\geq 1.5 \times 10^7$/kg of recipient weight.

Treatment Description:

Conditioning regimen:
Fludarabine 30 mg/m^2/day IV x 6 days (Day -7 to -2)
Melphalan 100 mg/m^2/day IV x 1 day (Day -1)
Total Body Radiation 200 cGy Day 0.

GVHD prophylaxis:
Sirolimus oral, target range 3-12 ng/ml
Tacrolimus oral or intravenous, target range 5-10 ng/ml

Double sequential cord blood transplantation will occur on Day 0

Routine post-transplant supportive care will be provided

Study Duration:

Patients will be followed for at least 24 months after transplantation

SCHEMA

	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0	Day +5
Fludarabine intravenous	√	√	√	√	√	√			
Melphalan intravenous							√		
TBI								√	
Start sirolimus oral					√				
Start tacrolimus intravenous					√				
Cord blood infusion								√	
Start G-CSF subcutaneous or intravenous									√

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1. OBJECTIVES

1.1 Study Design

This is a Phase II study designed to test the new conditioning regimen. One year significant viral infection rate will be compared to our historical control population, which has a one-year significant viral infection rate of 53%.

1.2 Primary Objectives

The primary objective of this Phase II trial is to determine the one year significant viral infection rate (viral infections requiring medical intervention) after double umbilical cord blood transplant using a novel conditioning regimen of fludarabine/melphalan/low dose total body radiation.

1.3 Secondary Objectives

Secondary objectives of this trial include:

- Time to neutrophil and platelet engraftment
- Rate of primary graft failure
- Rates of Gr. II-IV and Gr. III-IV acute GVHD at 100 days
- The rate of chronic GVHD
- 100-day treatment related mortality
- Immune reconstitution—CD 4 count at 12 months
- Relapse-free and overall survival at 1 and 2 years from transplantation
- Relapse Rate
- Rate of post transplant lymphoma
- Thrombopoieitin levels after transplant (optional correlate)

2. BACKGROUND

2.1 Stem Cell Transplant

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. Results of 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.[1] The Anthony Nolan 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.[2] Although these registries have grown to include over twelve 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.

2.1.1 Umbilical Cord Transplantation

Placental or umbilical cord blood (UCB) has been shown to contain sufficient progenitor cells for hematopoietic engraftment and can sustain hematopoiesis after transplantation between unrelated individuals.[3] Repositories of unrelated UCB collected from volunteer donors at the time of newborn delivery have been established in the United States and Europe.[4-6] An estimated 400,000 cord blood units are available world-wide, and can be shipped for immediate use.[7]

The first related cord blood transplant was performed in 1988, for a child with Fanconi's Anemia,[8] and the largest single institution UCB transplantation experience is at Duke University.[9] Rubinstein *et al* reported on the results of 562 cord blood transplants facilitated by the New York Blood Center. Younger age and a high total nucleated cell dose (TNC)/kg were associated with improved survival.[10] The European experience suggests a 30% disease-free survival in pediatric recipients of unrelated cord blood transplants, and again TNC dose/kg correlated with engraftment and survival.[11] Engraftment was improved with a stem cell dose greater than 3.7×10^7/kg infused. Since the average nucleated cell dose of a cord blood unit is approximately 1×10^9, most UCB units are only acceptable for children and small adults.[12]

Laughlin *et al* reported that single UCB units could restore hematopoiesis in adults after myeloablative transplant conditioning.[13] Sixty-eight patients, aged 18-55 years, with a median weight 69 kg, received a single UCB transplant. The median TNC dose at the time of transplantation was 1.6×10^7 TNC/kg. Neutrophil engraftment occurred at 27 days (range 13-59) and 8 patients died prior to engraftment. Platelet engraftment occurred at 58 days (range 35-142) in the 30 evaluable patients. As a result of this prolonged time to engraftment, the 100-day transplant related mortality was 41%, and only 18 patients were disease-free survivors forty months after transplantation.

Similar clinical experiences have been reported by Spanish,[14] and Eurocord,[15] and Japanese investigators.[16] In all studies, nucleated cell dose correlated with survival, and transplant-related mortality was high.

The ability to transplant adults and larger children safely with UCB blood is therefore limited by the number of hematopoietic progenitors contained in a single unit of UCB. In order to circumvent this problem strategies such as the co-transplantation of 2 or more UCB units have been proposed.

2.1.2 Double Umbilical Cord Transplantation

Early reports of patients infused with multiple, mismatched UCB units suggested that crossed immunologic rejection would not occur,[17] prompting the further examination of this technique. Recent studies of adult recipients have evaluated the outcome of transplantation of two cord blood units to adult recipients, thereby increasing the number of stem cells infused. At the University of Minnesota, 23 patients received two UCB units, thawed and administered sequentially, with a minimum combined cell dose of 3×10^7 TNC/kg. Cord blood units were a 4/6 HLA match with the patient and with each other. An ablative conditioning regimen of fludarabine, cyclophosphamide, and total body radiation was used. The median time to engraftment was 23 days, and mixed chimeric hematopoiesis was observed at day 21 in 24% of patients. Using cyclosporine and mycophenolate mofetil as graft versus host disease (GVHD) prophylaxis, the rate of Grade. II-IV acute GVHD was 65%. One year disease-free survival was 57%.[18]

More recently, the Minnesota group updated their clinical experience in a report of 110 individuals who underwent cord blood transplantation using a reduced intensity regimen containing low dose total body radiation (TBI).. Ninety-three patients received a double cord blood transplant; only those in whom a sufficiently large single unit was available received one unit. Ninety-two percent had neutrophil recovery with a median time to engraftment in those that engrafted of 12 days. Only 65% engrafted platelets by day 180. Grade II-IV acute GVHD occurred in 59% of patients, with chronic GVHD noted in 23% of patients. Transplant related mortality was 19% at 6 months, and 26% at 3 years. Event-free survival at 3 years was 38%, and there was an advantage to receiving two umbilical cord blood units.[19]

To build upon this double umbilical cord blood experience, two Phase I-II studies of double cord blood transplantation using the reduced intensity conditioning regimen of fludarabine, melphalan and antithymocyte globulin have now been completed at the Dana-Farber/Harvard Cancer Center (Protocols 03-061 and 05-154).[20,21] In these trials, 53 subjects received two UCB units, matched at 4/6 HLA loci or better to each other and to the recipient. GVHD prophylaxis regimens were cyclosporine and mycophenolate mofetil (03-061) or sirolimus and tacrolimus (05-154). Subjects received a combined total of 4.4×10^6 TNC/kg and 1.9×10^5 $CD34^+$ cells/kg pre-cryopreservation, without cohort differences. Engraftment kinetics did not differ based on GVHD prophylaxis, with neutrophil engraftment (absolute neutrophil count of 500) at a median of 21 days (13-70), and platelet engraftment (20 000/μL) at a median of 42 days (21-185). Five patients experienced graft loss between days 35 and 102. Transplant related mortality was 13% at 100 days, 24 % at one year, and 29% at two years. Grade II-IV acute GVHD occurred in 40% of patients treated with Cya/MMF and 9% of subjects treated with sirolimus/tacrolimus, $p = 0.035$. Chronic GVHD occurred in 39% of patients treated with cyclosporine/cellcept and 13% of patients treated

with sirolimus/tacrolimus. However, the relapse rate was higher in the sirolimus/tacrolimus group, 34% vs 14%, p = 0.05. Two year DFS was 55% for cyclosporine/MMF and 31% for sirolimus/tacrolimus, p=0.101. As a result of these two studies, double umbilical cord blood transplantation has become the standard at the DF/HCC, when UCB transplantation is required.

A preliminary study from our center compared survival after UCB using the conditioning regimen of melphalan, fludarabine, and antithymocyte globulin, with survival after a matched related donor or matched unrelated donor transplant, using a reduced intensity conditioning regimen. [22] Overall survival and progression-free survival was comparable among all three groups, with a 2 year overall survival of 50% and a 2 year progression-free survival of 34%. UCB patients had a higher transplant related mortality, mostly due to infection, but a decreased risk of relapse.

2.1.3 The Use of Low Dose Total Body Radiation in Cord Blood Conditioning

The Minnesota group has used a conditioning regimen of cyclophosphamide, fludarabine, and low dose total body radiation in over 100 patients.[19] The GVHD prophylaxis was cyclosporine and mycophenolate.Incidence of acute GVHD Grades II-IV was 59%. Transplant related mortality was 26% at three years. Viral infection rate was not analyzed in this study, but the risk of post transplant lymphoproliferative disorder was less without the use of antithymocyte globulin.[23] Compared to the DFCI/MGH experience on protocols 03-061 and 05-154, in Minnesota, the risk of graft versus host disease was greater but the incidence of second malignancies was less.[24] Our study outlined here utilizes low dose TBI to decrease the second malignancies and viral infections, but with the GVHD prophylaxis of sirolimus and tacrolimus to decrease GVHD. Our active cord blood protocol, 08-274, which is completing accrual, uses no TBI but a lower dose of ATG. However, that Phase I study is looking at ProstaglandinE2 incubation so the data is confounded.

Recently, the CIBMTR completed a Phase II study of reduced intensity double cord blood transplantation with the conditioning regimen of cyclophosphamide, fludarabine, and low dose total body radiation and a GVHD prophylaxis of cyclosporine or tacrolimus and mycophenolate mofetil..[25] Fifty-four patients were enrolled; preliminary data indicates a transplant related mortality of 20% at Day 180.

2.2 Viral Reactivation

Post transplant lymphoma remains a significant problem after our cord blood protocols.[24] The incidence of second cancers was 18% in the first 98 patients, with 16 post transplant lymphomas (PTLD), and 2 patients with donor derived MDS/MPD. There was a trend toward increased incidence in patients receiving antithymocyte globulin. The use of antithymocyte globulin in reduced intensity cord blood transplantation has been associated with PTLD in the Minnesota experience. [23] The incidence of EBV related complications was 21% with ATG and 2% in those

patients who did not receive ATG. Thus, a conditioning regimen without ATG may improve immune reconstitution and reduce late transplant related mortality. Interestingly, ATG used with myeloablative transplant had a much lower rate of EBV associated complications at 7%. The use of ATG was associated with a lower risk of GVHD. Reactivation of other viruses was not addressed in this study.

In addition to EBV infections, other viral infections remain a significant cause of morbidity and mortality in the first year post transplant. We examined the viral infection rate in the 53 patients treated above, focusing on viral infections that required medical intervention in the first year. In these patients, there were 28/53 viral infections requiring intervention or 53%. The infections were as follows: 11 CMV, 9 EBV, 2 adeno, 2 HHV-6, and 4 zoster.

The etiology of the increased infection rate after cord blood transplantation is complex, and includes factors such as a lower CD34+ dose/kg infused and qualitative differences in the lymphocytes in cord blood. For example, T lymphocytes from cord blood are CD45RA+ and express low levels of activation markers.[26] Expression of the chemokine receptor CCR7 may be important for immune recovery post transplant. Higher levels of CCR7+ CD4+ T cells at Day 30 post transplant was associated with prolonged survival.[27]

A variety of studies have reported increased viral infection after UCB transplantation. One hundred and fourteen children who were seropositive for varicella zoster virus underwent either cord blood transplant (n=37) or T replete bone marrow transplant (n=77).[28] Thymoglobulin was given for all cord patients, and 22 bone marrow patients who received an unrelated graft. Visceral dissemination occurred in 1 bone marrow patient and 6 cord blood patients (p=0.0005). CD4+ counts were lower after cord blood transplant. Parkman and colleagues have shown that children with a positive antigen response to herpes viruses post UCB transplantation had fewer infections and decreased relapse.[29] In a combined pediatric and adult study, MD Anderson has reported a 40% incidence of viral infections, in patients treated with a variety of conditioning regimens.[30]

Cytomegalovirus reactivation remains a significant cause of morbidity after cord blood transplantation. Our center, using fludarabine, melphalan, and thymoglobulin conditioning, reported that clearance of CMV viremia depends on the recovery of C4+CD45RA+ T cells and T cell receptor rearrangement excision circles (TREC) levels of greater than 2000 copies/ug DNA.[31] Survival was improved in patients that attained TREC levels greater than 2000 copies/ug DNA. The Spanish group, using antithymocyte based conditioning, reported a risk of CMV infection of 59% and CMV disease of 9% using prophylactic valganciclovir.[32] The Japanese group, using fludarabine based regimen, reported an incidence of positive CMV antigenemia of 55% and 16% of CMV disease.[33] CMV enterocolitis was seen in the majority of cases with CMV disease, and graft versus host disease was a risk factor for the development of CMV disease.

Double UCB transplantation is associated with a faster time to neutrophil engraftment and decreased relapse.[19,34] It is not clear if the viral infection rate, however, is decreased after double UCB compared to single UCB. The French group studied 31 adult UCB patients, of whom 27 received a double UCB transplant.[35] Patients received a variety of conditioning regimens, of which some contained antithymocyte globulin. The incidence of recurrent CMV infection was 21%; there were 2 deaths from EBV lymphoproliferative disorder and 1 from adenovirus.

Several centers have compared infections after UCB transplantation to those seen after transplantation with other graft sources.[36] Forty-eight recipients of myeloablative, single UCB transplantation were compared with 144 unrelated bone marrow/peripheral blood stem cell transplant recipients. The UCB patients had a higher risk of early bacterial infection but the 100 day and 3 year incidence of infection related mortality was similar. All cord blood patients received a myeloablative total body radiation conditioning regimen. In our center, progression-free survival was similar among recipients of reduced intensity double UCB, matched related donor, and matched unrelated donor transplants, but the transplant related mortality after Day 100 was higher in the UCB recipients.[22] The two year transplant related mortality was 29% for UCB receiving fludarabine, melphalan, and antithymocyte globulin, 9% for unrelated donor, and 8% for matched related donor. Infection was the leading cause of late transplant related mortality.

In this study, to improve immune reconstitution and decrease post transplant viral infection, we will substitute low dose total body radiation at a dose of 200 cGy for the antithymocyte globulin in the conditioning regimen. The fludarabine and melphalan doses will remain the same. We will compare one year significant viral infection rates requiring intervention to our historical control population who were treated with fludarabine, melphalan, and antithymocyte globulin. GVHD may be higher with this regimen and therefore we will utilize the GVHD prophylaxis regimen of sirolimus and tacrolimus that has proven to control acute GVHD at DF/HCC across all types of transplants and conditioning regimens.

2.3 Platelet Engraftment and Thrombopoietin

Platelet engraftment is delayed after cord blood transplant, with a median day to platelet count >20 x 10 (9)/L of 42 days, range 21-185 days. The death rate from CNS bleeding is 1-2%. Delayed platelet engraftment is also an important contributor to the increased morbidity, poor quality of life, and cost after double cord blood transplant. Therefore, as an optional correlate, we will study thrombopoietin (TPO) levels after cord blood transplant, to determine if further investigation of TPO receptor agonists post cord blood transplant is warranted.

The thrombopoietin (TPO) receptor, c-Mpl, is a hematopoietic cytokine receptor and TPO receptors on hematopoietic stem cells and platelets.[387] When platelet production is low, circulating levels of TPO rise.[39]

Two recombinant TPO molecules (rhTPO and PEG-rHuMDGF) were developed in the late 1990's after the successful cloning of human TPO. Unfortunately, paradoxically, some patients developed thrombocytopenia, and there was no improvement in time to platelet recovery in patients undergoing hematopoietic stem cell transplantation.[40]

Recently, two thrombopoietin receptor agonists, romiplostim (N-Plate) and eltrombopag, have been approved for patients with immune thromobocytopenia. Romiplostim is composed of four peptides that bind to the thrombopoietin receptor cMPL fused to an Fc fragment.[41] Its effectiveness in ITP was shown in two Phase III studies; a platelet count of >50 x 10 (9)/L was seen in 61% of non splenectomized and 38% splenectomized patients receiving romiplostim and in <1% of placebo patients. [41] Side effects have included rebound thrombocytopenia after drug discontinuation and increased bone marrow reticulum. [42] A recent randomized study showed that patients treated with romiplostim have a higher rate of platelet response, fewer blood transfusions, and a higher quality of life than patients treated with the standard of care.[43] In patients with myelodysplasia receiving romiplostim, there was a transient increase in circulating blasts, but no increase in transformation to AML.[42]

To understand the changes in TPO levels after double cord blood transplant so as to ascertain the potential benefit of a future clinical trial with romiplostim, we will collect TPO levels after cord blood transplant, as described in Section 8.2. and Appendix 1. Participation in the TPO level portion of the study will be optional for patients. TPO levels will be determined with the assistance of Dr. David Kuter and Dr. Robert Makar and TPO levels will be measured pre conditioning, Day 0, Day 1 after transplant, weekly until Day 60, Day 100, Day 180, Day 360. TPO levels will also be measured pre platelet transfusion, 1 hour, 6 hours and 24 hours post platelet transfusion for the first 3 platelet transfusions given Monday through Thursday post transplant. TPO levels will be correlated with complete blood counts, and platelet counts and platelet transfusions, and a timeline of TPO levels post transplant will be formulated.

2.4 Rationale

DUCBT appears to reduce the incidence of early treatment related mortality when compared with single UCB transplantation, however, there remains a high risk of viral infection, with the use of antithymocyte globulin. This study will test a non antithymocyte globulin conditioning regimen with the use of fludarabine, melphalan, and low dose total body radiation. Low dose total body radiation has been used safely in several hundred adult patients undergoing double cord blood transplantation.[23,25] Viral infection rate was not well analyzed in these studies. Sauter et al have recently reported on viral infection in a conditioning regimen without ATG.[44] Patients in this study received either ablative (n=52) or nonmyeloablative (n=20) conditioning regimens followed by double cord blood transplant with cyclosporine and mycophenolate mofetil prophylaxis.Thirty percent of patients had a serious viral infection between Days 31-60 after transplant. CMV accounted for 45% of the viral infections.

We hypothesize a 20% reduction in clinically significant viral infections with the elimination of antithymocyte globulin. We will monitor for graft failure and graft versus host disease. While it is possible that GVHD may be increased, the risk of GVHD with our current ATG containing regimen and GVHD prophylaxis of sirolimus and tacrolimus is low. We would not anticipate a higher risk of relapse without ATG; based on the Minnesota experience[19] If successful, this trial will increase the safety of UCB transplantation in adults, by reducing the risks of treatment-related mortality due to viral infection.

Selection of Primary Endpoint:
Due to advances in cord blood transplant, such as the use of double cord blood transplant, engraftment has improved and thus engraftment is not a clinically meaningful endpoint in adult double cord blood transplant in 2011. Transplant related mortality (TRM) is low in the first 100 days post transplant, at <15% in our earlier protocols with ATG. [20,21]This 100 day TRM would reflect engraftment issues. However, the 3 year TRM is 29% which reflects late viral infections and second cancers.[22,24] Thus, we feel that the viral reactivation is a more clinically meaningful endpoint based on our earlier experience. However, we include an interim analysis for the primary endpoint and at that time we will evaluate the neutrophil engraftment in parallel.

Interim Analysis:
As of February 12, 2013, 16 patients were enrolled, among whom 14 patients were transplanted. Of these 14, data for the primary endpoint was available for the first 13 patients, and these 13 patients are included in the interim analysis. Of these 13, 8 patients experienced clinically significant infections (5 HHV-6, 2 CMV, 1 both CMV and HHV-6), 1 patient experienced graft failure, 1 patient experienced grade III-IV acute GVHD and 1 patient died of TRM within day 100 of transplantation. Since the number of infections exceeds the threshold of 7, as specified in the original design, accrual was temporarily suspended and the results of the interim analysis reviewed with the DSMC. The team reviewed the data and has made the following adjustments. A more aggressive antiviral prophylaxis, monitoring, and treatment plan is outlined in the revised protocol. In addition, new stopping rules are outlined in the statistics.

Second interim analysis:
As of As of October 14, 2014, 11 additional patients were transplanted after the study was re-opened. Among the first 10 patients who were subsequently enrolled, 5 patients experienced clinically significant infections (3 CMV, 1 CMV and Herpes Zoster, 1 Adenovirus and HHV-6), therefore the study met the early stopping rule which was specified in the amendment following the first interim analysis (≥5 clinically significant infections in 10 subsequently enrolled patients).

The accrual to the study was temporarily suspended and the study team met on December 16, 2014. The results of the interim analysis were reviewed with the DSMC. After reviewing additional outcome data, the study team felt that although the overall targeted reduction in infection rates will not be reached, the outcome was good among the 10 patients who were subsequently enrolled since the study re-opened with a more restricted age eligibility of under 65: no 100 day TRM, 17% TRM and 83% survival at 6 months, respectively. The study team

suggested to re-open the study and to complete the accrual so more data can be gathered within the group with age under 65 (7 more patients need to be accrued).

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

3.1.1 Participants with hematologic malignancies or hematologic disorders for whom allogeneic stem cell transplantation is deemed clinically appropriate. Eligible diseases and stages include:

1. Non-Hodgkin's lymphoma, or Hodgkin's lymphoma in 2nd or subsequent complete remission or in partial remission with documented chemosensitivity to the most recent chemotherapy regimen. Prior autologous transplantation is required, unless deemed medically inappropriate by the treating physician.
2. Multiple myeloma: relapsed but with chemosensitive disease. Bone marrow plasma cells may not exceed 20% of the total cellularity.
3. Chronic lymphocytic leukemia: Any Rai stage III or IV, lymphocyte doubling time of 6 months, or stage I-II with progression after $\geq$ 2 chemotherapy regimens, in partial remission with documented chemosensitivity to the most recent chemotherapy regimen.
4. Acute myelogenous or acute lymphoblastic leukemia in second or subsequent complete remission or in first remission with adverse cytogenetic/molecular features or a documented antecedent hematologic disorder.
5. Myelodysplastic disorder
6. Myeloproliferative disorder including myelofibrosis, chronic myelogeneous leukemia resistant to tyrosine kinase inhibitorsAplastic anemia with no response to immunosuppressive therapy.

3.1.2 Patient must be appropriate for reduced intensity regimen, according to the treating physician.

3.1.3 Lack of 6/6 or 5/6 HLA-matched related, 8/8 HLA-matched unrelated donor, or unrelated donor not available within a time frame necessary to perform a potentially curative stem cell transplant.

3.1.4 Age 18 – 65 years

3.1.5 ECOG performance status of 0-2

3.2 Exclusion Criteria

Participants who exhibit any of the following conditions at screening will not be eligible for admission into the study.

3.2.1 Cardiac disease: symptomatic congestive heart failure or evidence of left ventricular dysfunction (Ejection fraction < 40%) as measured by gated radionucleotide ventriculogram or echocardiogram; active angina pectoris, or uncontrolled hypertension.

3.2.2 Pulmonary disease: symptomatic chronic obstructive lung disease, symptomatic restrictive lung disease, or corrected DLCO of < 50% of predicted, corrected for hemoglobin.

3.2.3 Renal disease: serum creatinine > 2.0 mg/dl.

3.2.4 Hepatic disease: serum bilirubin > 2.0 mg/dl (except in the case of Gilbert's syndrome or ongoing hemolytic anemia), SGOT or SGPT > 3 x upper limit of normal.

3.2.5 Neurologic disease: symptomatic leukoencephalopathy, active CNS malignancy or other neuropsychiatric abnormalities believed to preclude transplantation

3.2.6 HIV antibody. HIV-positive individuals are at increased risk of lethal infections when treated with marrow-suppressive therapy.

3.2.7 Uncontrolled infection

3.2.8 Pregnancy or breast feeding mother.

3.2.9 Inability to comply with the requirements for care after allogeneic stem cell transplantation

3.3 Inclusion of Women, Minorities and Other Underrepresented Populations

Women and minorities are included in the eligibility criteria. Because non Caucasian populations have a difficult time finding matched adult volunteer donors, umbilical cord blood transplantation is an important alternative stem cell source for minority patients. We will attempt to find appropriately matched cord blood donors for all eligible patients, regardless of gender, race, or ethnicity. Gender, race and ethnicity will be recorded in our QACT database, per standard transplant procedures.

3.4 Donor Selection

Cord blood donors are enrolled by a number of cord blood banks in the United States and worldwide. Donors undergo a strict evaluation according to local cord blood bank

practices and are consented by the local cord blood bank. Enrollment and consent of cord blood donors are not covered in this protocol.

3.5 Selection of Cord Blood Units

3.5.1 The patient and the cord blood units must be a 4/6 HLA A, B, DRβ1 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

3.5.2 Determination of histocompatibility will be made by molecular typing of HLA class I and class II alleles. Cord unit to patient matching should be at the allele level (high resolution) whenever possible. 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 one the Dana Farber/Harvard Cancer Center sites.

3.5.3 Total combined nucleated cell dose from the 2 cord blood units must be $\geq 3.7 \times 10^7$ TNC/kg (pre-cryopreservation). Each single cord blood unit cell dose must be $\geq 1.5 \times 10^7$ TNC/kg (pre-cryopreservation).

3.5.4 Choice of cord blood units, when multiple suitable units are available will use the following algorithm:

- Higher cell dose triumphs over HLA match when TNC is $\leq 2.0 \times 10^7$ NC/kg for each single cord blood unit
- Closer HLA match triumphs over cell dose when TNC is > 2.0 x NC/kg for each single cord blood unit unless there is a >50% higher cell dose in the less well matched cord blood unit
- For selection between equivalent units choose:
  CD34+ dose (higher dose preferable)
  Greater viability (never use a unit with viability less than 90%)

3.5.5 Cord blood units to which the patient has preformed HLA A, B, C, DR antibodies cannot be used.

3.5.6 Cord units must not be HLA identical, unless a 6/6 match with the patient.

3.5.7 Double mismatches at any given locus should be avoided

Please seen appendix 5 for more details.

3.6 Evaluation, Counseling and Patient Consent

Patients are referred to the Dana-Farber/Harvard Cancer Center (DF/HCC) for consideration of allogeneic stem cell transplantation. Patients are completely evaluated and presented at a group conference where the transplant team will suggests a course of treatment. In the appropriate clinical setting, umbilical cord blood transplantation is

suggested. The patient then undergoes a thorough pre-transplant evaluation including a 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 family before transplantation. 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 some aspects of cord blood transplantation are considered experimental. Consent is obtained using forms approved by the Dana Farber Institutional Review Board.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants with the DF/HCC Quality Assurance Office for Clinical Trials (QACT) central registration system. Registration must occur prior to the initiation of therapy. Any participant not registered to the protocol before treatment begins will be considered ineligible and registration will be denied.

A member of the study team will confirm eligibility criteria and complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol treatment. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a participant does not receive protocol therapy following registration, the participant's protocol status must be changed. Notify the QACT Registrar of participant status changes as soon as possible.

4.2 Registration Process for DF/HCC and DF/PCC Institutions

The QACT registration staff is accessible on Monday through Friday, from 8:00 AM to 5:00 PM Eastern Standard Time. In emergency situations when a participant must begin treatment during off-hours or holidays, call the QACT registration line at [redacted] and follow the instructions for registering participants after hours.

The registration procedures are as follows:

1. Obtain written informed consent from the participant prior to the performance of any study related procedures or assessments.

2. Complete the protocol-specific eligibility checklist using the eligibility assessment documented in the participant's medical/research record. **To be eligible for registration to the study, the participant must meet each inclusion and exclusion criteria listed on the eligibility checklist.**

Reminder: Confirm eligibility for ancillary studies at the same time as eligibility for the treatment study. Registration to both treatment and ancillary studies will not be completed if eligibility requirements are not met for all studies.

3. Fax the eligibility checklist(s) and all pages of the consent form(s) to the QACT at [redacted].

   Exception: DF/PCC Affiliate sites must fax the entire signed consent form including HIPAA Privacy Authorization and the eligibility checklist to the Network Affiliate Office. The Network Affiliate Office will register the participant with the QACT.

4. The QACT Registrar will (a) validate eligibility, (b) register the participant on the study, and (c) randomize the participant when applicable.

5. The QACT Registrar will send an email confirmation of the registration and/or randomization to the person initiating the registration immediately following the registration and/or randomization.

4.3 General Guidelines for Other Participating Institutions

N/A

4.4 Registration Process for Other Participating Institutions

N/A

5. TREATMENT PLAN

Treatment will be administered on an inpatient basis. Expected toxicities and potential risks for melphalan, fludarabine, and low dose total body radiation are described in Section 6 (Expected Toxicities and Dosing Delays/Dose Modification). No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Day 0 corresponds to the day of cord blood infusion.

Table 1

	Day -7	Day -6	Day - 5	Day -4	Day -3	Day -2	Day -1	Day 0	Day +5
Fludarabine 30mg/m^2/day IV	√	√	√	√	√	√			
Melphalan 100mg/m^2/day IV							√		

TBI 200 cGy								√	
Start sirolimus PO					√				
Start tacrolimus IV or PO					√				
Start GCSF IV or SC									√

5.1 Conditioning Therapy

Chemotherapy and GVHD prophylaxis medication doses will be based on actual weight in all circumstances. Administer chemotherapeutic agents according to institutional policies and procedures. There are no dose reductions in the conditioning therapy.

5.1.1 Fludarabine will be administered intravenously at a dose of 30mg/m^2/day on days -7, -6, -5, -4, -3, and -2 (total dose 180mg/m^2)

5.1.2 Melphalan will be administered intravenously at a dose of 100mg/m^2/day on day -1.

5.1.3 Total Body Irradiation 200 cGy in 1 fraction on Day 0 at least 2 hours prior to cord blood infusions. Radiation sources and dose rates will be per institutional standards.

5.2 GVHD Prophylaxis

GVHD prophylaxis will begin on Day -3

5.2.1 Tacrolimus

Tacrolimus (FK-506, Prograf) will be administered at a starting dose of 0.02 mg/kg intravenously by continuous infusion. Subsequent dosing will be based on clinical toxicity, GVHD, concurrent medications, medical conditions, prior drug levels, drug-drug interactions and current blood levels. The target serum concentration is 5-10 ng/ml. The oral formulation may be substituted at the treating physician's discretion. Doses may be re-administered if the subject vomits within 15 minutes of the dose, or if capsule fragments are visible in the vomitus. In the absence of GVHD and at the discretion of the attending physician, tacrolimus will begin to be tapered at approximately day +100 after transplantation, with the goal of discontinuation by 6-9 months post transplant.

5.2.2 Rapamycin

Rapamycin (Sirolimus, Rapamune) will be given as an oral loading dose of 12 mg on day -3, followed by a daily oral dose of 4 mg/day. Doses may be re-administered if the subject vomits within 15 minutes of the dose, or if pill fragments are visible in the vomitus. Subsequent dosing will be based on clinical toxicity, GVHD, concurrent medications, medical conditions, prior drug levels, drug-drug interactions and current blood levels. Many medicines, including fluconazole,

can affect sirolimus levels. Patients on fluconazole should receive a loading dose of 12 mg followed by a daily oral dose of 2 mg/day. Patients should not start fluconazole until Day 0. The target serum concentration of sirolimus is 3-12 ng/ml. In the absence of GVHD and at the discretion of the attending physician, sirolimus will begin to be tapered at approximately day +100 after transplantation, with the goal of discontinuation by 6-9 months post transplant

5.3 Umbilical Cord Blood Unit Processing and Infusion

5.3.1 Cord Blood Thawing Procedure

Cells are mixed with a hypertonic solution of Dextran 40 + 5% albumin (Dextran/albumin) immediately upon thawing. Cells are then washed to remove DMSO, free hemoglobin and other cellular products, as per institutional practices

5.3.2 Release of Cord Blood from the Cell Processing Facility

All subjects will receive two cord blood units, administered sequentially, no less than 2 hours and no more than 5 hours apart. Cord blood units must not be released until after total body radiation is complete on Day 0. The times of release from the stem cell processing facility for the cord blood units should be recorded.

Umbilical cord blood units will always be released and administered according to the following sequence. The larger cord blood unit, as measured by the pre-cryopreservation TNC/kg, will be administered first. If the larger cord blood unit is within 105% of the size of the smaller cord blood unit, the better HLA-matched unit will be administered first. HLA-A, B and DRβ1 will be considered for matching, with preference to better DRβ1 matching, should both cord blood units have equivalent numbers of mismatches. In the event that both cord blood units are the same size and HLA match, the cord blood unit collected most recently will be administered first.

5.3.3 Administration of Cord Blood Units

Cord blood units will be administered according to institutional policies and procedures. If possible, if a cord blood segment is available, the cord blood unit will be tested for HHV-6 by PCR.

5.4 Post transplant Supportive Care

5.4.1 Infection Prophylaxis: At a minimum, the following practices will be observed: prophylactic antibiotics (such as levoquin or other appropriate agent) against gram negative organisms; ; and antifungal prophylaxis with fluconazole (or another appropriate agent). Patients on fluconazole should have a dose reduction in sirolimus as per Section 5.2.2. Fluconazole should not start until Day 0. Voriconazole should not be used as primary prophylaxis. Antifungal prophylaxis should continue through at least hospital discharge. Prophylaxis against *Pneumocystis jirovecii* will begin upon hospital admission through the day prior to stem cell transplantation using trimethoprim-sulfamethoxazole (unless contraindicated due to allergy), and will resume upon hospital discharge. Atovaquone is the preferred agent at all times for trimethoprim-sulfamethoxazole allergic patients and for all subjects at the time of discharge

5.4.2 Extended viral monitoring will include monitoring for Cytomegalovirus (CMV), Epstein-Barr virus and Human Herpesvirus-6. CMV will be treated pre-emptively. Rising EBV titers on two sequential assays may be treated pre-emptively with rituximab to prevent post-transplant lymphoproliferative disorder.

5.4.3 Recombinant Granulocyte Colony Stimulating Factor (G-CSF)-either Neupogen or Granix will be administered intravenously or subcutaneously from day +5 until engraftment of neutrophils (absolute neutrophil count of 1000 cells/μL for 2 consecutive days). Thereafter, G-CSF will be administered at the treating physician's discretion. G-CSF dosing is as follows: patients ≤ 65 kg receive 300 mcg/day, patients 66-100 kg receive 480 mcg/day, and patients > 100 kg receive 600 mcg/day.

5.4.4 Transfusion support per Dana-Farber/Harvard Cancer Center guidelines will be provided.

5.4.5 Central venous access will be obtained on, or prior to, the date of admission.

5.4.6 Hyperalimentation (TPN) will be provided at the treating physician's discretion.

5.4.7 Supplemental intravenous immunoglobulin will be transfused at the treating physician's discretion. It is recommended that plasma immunoglobulins be measured at monthly intervals (at a minimum) and replacement be considered for levels less than 400 mg/dL.

5.4.8 After hospitalization, subjects will be followed closely to monitor for complications related to transplantation. Patients will receive standard post-transplant discharge teaching and guidelines to prevent infection. All patients will receive the same treatment as outlined above. There will be no dose adjustments for chemotherapy.

5.4.9 In an effort to reduce the risk of serious HHV-6 infection, the following antiviral prophylaxis will be instituted during conditioning: Famvir 500 mg tid or Valtrex 1 gram daily unti at least Day +30 after transplant. If patients cannot take oral medications, then Acylovir 5 mg/kg IV every eight hours will be instituted. Dose may be rounded. Antiviral prophylaxis, such as Famvir 500 mg daily or Acyclovir 400 mg bid will continue through the longer of 1 year or until the discontinuation of all immunosuppression. Doses may be adjusted based on toxicity and renal dysfunction at the discretion of the investigator. A suggested management plan for HHV-6 reactivation is included in Appendix 6.

5.5 Duration of Therapy

Duration of therapy will be as outlined in the protocol. There will be no adjustments in conditioning regimen, as per standard transplant care.

5.6 Duration of Follow Up

Participants will be followed for at least 24 months post transplantation. Patients removed from the study will continue to be followed for survival.

5.7 Criteria for Removal from Study

Study subjects may withdraw their consent to participate in this trial at any time. Once withdrawn, the study subject will receive post-transplant care considered standard and routine for their individual case.
Subjects may be removed from study treatment 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 or progressive disease, second transplantation)

The reason for study removal and the date the participant was removed must be documented in the study-specific case report form (CRF). Alternative care options will be discussed with the participant.

In the event of unusual or life-threatening complications, participating investigators must immediately notify the Principal Investigator: Dr. Zachariah DeFilipp at [redacted].

5.8 Suggested Guidelines for Initiation of Treatment of Significant Viral Infections:

1. Cytomegalovirus (CMV): Patients with CMV infection, such as documented CMV from bronchoscopy or gastrointestinal biopsy, should be treated. Patients with CMV reactivation on 2 consecutive testing (CMV antigenemia >3 cells or positive CMV DNA) should be treated. Treatment may be ganciclovir, valganciclovir, foscarnet, or lymphocyte preparations (for example, third party Cytotoxic T lymphocytes) as determined by the treating investigator.
2. Epstein Barr Virus (EBV): Patients with documented EBV lymphoproliferative disorder, such as on lymph node biopsy, should be treated. Patients with 2 consecutive EBV DNA >1000 should also be treated. Treatment may be rituximab, chemotherapy, or lymphocyte preparation as determined by the treating investigator..
3. Human Herpes Virus 6 (HHV6): Suggested guidelines are given in Appendix 6..
4. Adenovirus: Patients with documented adenovirus in the lung should be treated. Patients with adenovirus in the urine should be treated if there is painful hemorrhagic cystitis, not relieved with IV fluids or continuous bladder irrigation. Treatment may be cidofovir or lymphocyte preparations, as determined by the treating investigator.
5. Herpes Zoster: Patients with Herpes Zoster infection (shingles) should be treated. Treatment may be high dose acyclovir.

6. EXPECTED TOXICITIES AND DOSING DELAYS/DOSE MODIFICATIONS

CTEP Version 4 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. The CTEP Active Version of the CTCAE is identified and located on the CTEP website at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

All appropriate treatment areas should have access to a copy of the CTEP Active Version of CTCAE.

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Anticipated Toxicities

6.1.1 Adverse Event Lists(s) for Fludarabine

The most common adverse events include myelosuppression, fever and chills, and nausea and vomiting. Other commonly reported events include malaise, fatigue, anorexia and weakness. Serious opportunistic infections have occurred in patients with CLL treated with fludarabine and are due to myelosuppression and prolonged impairment of cell mediated immunity.

Peripheral neuropathy has been reported although would be unusual following a single course of therapy. CNS toxicity is rare following a single course of therapy (at a dose of 125mg/m^2). Possible manifestations include delirium, seizures, coma, and visual disturbances due to optic neuritis. Other rare toxicities include hemolytic anemia, respiratory distress,

6.1.2 Adverse Event List(s) for Melphalan

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.

Rare side effects include hypersensitivity reaction and skin necrosis.

6.1.3 Adverse Event List for Total Body Irradiation

TBI can cause nausea, vomiting, diarrhea, skin rash, mucositis, neutropenia, fever. Late effects include liver damage, kidney damage, pneumonitis, cataracts, vertebral damage, sterility, and second cancers.

6.1.4 Adverse Event List for G-CSF

Toxicities to G-CSF commonly include: mild to moderate bone pain, myalgia, vomiting, fatigue, headache, insomnia, Pain at the site of the injection, and fever.

There are rare reports of splenic rupture in healthy individuals receiving G-CSF to mobilize stem cells for transplantation. Other rare side effects include acute respiratory distress syndrome and alveolar hemorrhage.

6.1.5 Adverse Event List for Tacrolimus

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, hypokalemia, tremor, and neurologic toxicity may occur. There is an increased risk of opportunistic infections and secondary malignancies.

Rare side effects include seizure, posterior reversible encephalopathy. Many drugs, such as antifungal agents and calcium channel blockers, may alter tacrolimus levels.

6.1.6 Adverse Event List for Sirolimus

The primary toxicities of sirolimus are hypertriglyceridemia, hypercholesterolemia, mild thrombocytopenia, anemia, leukopenia, hypokalemia, elevated LDH, arthralgia, epistaxis, edema, and infections. Clinically significant elevations in hepatic transaminases without sequelae have been noted and there is an increased incidence of veno-occlusive disease of the liver in the myeloablative setting, but not with this reduced-intensity conditioning regimen. A syndrome of thrombotic microangiopathy, comprised of microangiopathic hemolytic anemia, thrombocytopenia and renal dysfunction has been described in association with sirolimus and tacrolimus use. Sirolimus may increase the risk of opportunistic infections and post-transplant lymphoproliferative disorders.

Rare side effects include hypersensitivity reactions, proteinuria, and dermatitis.

6.2 Toxicity Management:

Significant toxicity is expected in this cord blood transplant protocol. Patients will receive supportive care and medical management of toxicity.

6.3 Dose Modifications/Delays:

There will be no dose modifications or delays in the conditioning regimen treatment. Sirolimus and tacrolimus will be dosed based on blood level, as indication in Section 5.2. Sirolimus and tacrolimus may be modified based on clinical toxicity, and alternative immunosuppression used, at the discretion of the treating investigator.

7. DRUG FORMULATION AND ADMINISTRATION

7.1 Fludarabine

7.1.1 **Description**

FLUDARA FOR INJECTION contains fludarabine phosphate, a fluorinated nucleotide analog of the antiviral agent vidarabine, 9-_-D-arabinofuranosyladenine (ara-A) that is relatively resistant to deamination by adenosine deaminase. Each vial of sterile lyophilized solid cake contains 50 mg of the active ingredient fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Reconstitution with 2 mL of Sterile Water for Injection USP results in a solution containing 25 mg/mL of fludarabine phosphate intended for intravenous administration. The chemical name for fludarabine phosphate is 9H-Purin-6-amine, 2-fluoro-9-(5-0-phosphono-_-D-arabinofuranosyl) (2-fluoro-ara-AMP). The molecular formula of fludarabine phosphate is C10H13FN5O7P (MW 365.2)

7.1.2 **Form**

FLUDARA FOR INJECTION is supplied as a white, lyophilized solid cake. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol, and sodium hydroxide to adjust pH to 7.7. The pH range for the final product is 7.2-8.2. Store under refrigeration, between 2°-8°C (36°-46°F).

FLUDARA FOR INJECTION is supplied in a clear glass single dose vial (6 mL capacity) and packaged in a single dose vial carton in a shelf pack of five. NDC 50419-511-06

7.1.3 **Storage and Stability**

Store intact vials under refrigeration at 2°C to 8°C (36°F to 46°F). Reconstituted vials are stable for 16 days at room temperature of 15°C to 30°C (59°F to 86°F) or refrigerated, although the manufacturer recommends use within 8 hours due to no preservatives. Solutions diluted in saline or dextrose are stable for 48 hours at room temperature or under refrigeration.

7.1.4 **Compatibility**

Stable in D_5W, NS, sterile water for injection.

7.1.5 **Handling**

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

7.1.6 **Availability**

Fludarabine is commercially available.

Manufactured by: Ben Venue Laboratories, Bedford, OH 44146
Manufactured for: Teva Pharmaceuticals

7.1.7 Preparation

Reconstitute vials with SWI, NS, or D_5W to a concentration of 10-25 mg/mL. Standard I.V. dilution: 100-125 mL D_5W or NS.

7.1.8 Administration

Fludarabine is administered intravenously as a bolus over 30 minutes or as per institutional guidelines

7.2 Melphalan

7.2.1 Description

Melphalan, also known as L-phenylalanine mustard, phenylalanine mustard, L-PAM, or L-sarcolysin, is a phenylalanine derivative of nitrogen mustard. Melphalan is a bifunctional alkylating agent that is active against selected human neoplastic diseases. It is known chemically as 4-[bis(2-chloroethyl)amino]-*L*-phenylalanine. The molecular formula is C13H18Cl2N2O2 and the molecular weight is 305.20. Melphalan is the active L-isomer of the compound and was first synthesized in 1953 by Bergel and Stock; the D-isomer, known as medphalan, is less active against certain animal tumors, and the dose needed to produce effects on chromosomes is larger than that required with the L-isomer. The racemic (DL-) form is known as merphalan or sarcolysin. Melphalan is practically insoluble in water and has a pKa1 of 2.5.

7.2.2 Form

ALKERAN for Injection is supplied in a carton containing one single-use clear glass vial of freeze-dried melphalan hydrochloride equivalent to 50 mg melphalan and one 10-mL clear glass vial of sterile diluent (NDC 0173-0130-93).

7.2.3 Storage and Stability

Store intact vials at controlled room temperature 15° to 30°C (59° to 86°F) and protect from light**.** . Complete administration within 60 minutes of reconstitution.

7.2.4 Compatibility

Stable in NS

7.2.5 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and

safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

7.2.6 **Availability**

Melphalan is commercially available

7.2.7 **Preparation**

The time between reconstitution/dilution and administration of parenteral melphalan must be kept to a minimum (manufacturer recommends <60 minutes) because reconstituted and diluted solutions are unstable. Dissolve powder initially with 10 mL of supplied diluent to a concentration of 5 mg/mL; shake immediately and vigorously to dissolve. Immediately dilute dose in NS to a concentration of ≤0.45 mg/mL (manufacturer recommended concentration). Do not refrigerate solution; precipitation occurs. The manufacturer recommends administration within 60 minutes of reconstitution.

7.2.8 **Administration**

Melphalan is administered intravenously as a bolus over 15 minutes or as per institutional guidelines

7.3 Tacrolimus

7.3.1 **Description**

Tacrolimus is available for oral administration as capsules containing the equivalent of 0.5 mg, 1 mg or 5 mg of anhydrous tacrolimus. Inactive ingredients include lactose, hydroxypropyl methylcellulose, croscarmellose sodium, and magnesium stearate. The 0.5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide, the 1 mg capsule shell contains gelatin and titanium dioxide, and the 5 mg capsule shell contains gelatin, titanium dioxide and ferric oxide.

Tacrolimus is also available as a sterile solution containing the equivalent of 5 mg anhydrous tacrolimus in 1 mL for administration by intravenous infusion only. Each mL contains polyoxyl 60 hydrogenated castor oil (HCO-60), 200 mg, and dehydrated alcohol, USP, 80.0% v/v. Prograf injection must be diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection before use.

7.3.2 **Form**

Tacrolimus is available for oral administration as capsules in strengths of 0.5 mg, 1 mg or 5 mg. Injection is available as solution containing 5mg in 1 ml.

7.3.3 **Storage and Stability**

Injection: Prior to dilution, store at 5°C to 25°C (41°F to 77°F). Following dilution, stable for 24 hours in D_5W or NS in glass or polyethylene containers.

Capsules: Store at room temperature of 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F)..

7.3.4 **Compatibility**

Stable in NS or D5W

7.3.5 **Handling**

Use appropriate precautions for handling and disposal

7.3.6 **Preparation**

Dilute with 5% dextrose injection or 0.9% sodium chloride injection to a final concentration between 0.004 mg/mL and 0.02 mg/mL

7.3.7 **Administration**

Tacrolimus will be administered intravenously as a continuous infusion or orally at the discretion of the investigator

7.4 Sirolimus

7.4.1 **Description**

Sirolimus is indicated for the prevention acute graft-versus-host disease (GVHD) in allogeneic stem cell transplantation; treatment of refractory acute or chronic GVHD, prophylaxis for organ rejection in patients receiving renal and heart transplants.

Inactive ingredients in RAPAMUNE Oral Solution are: Phosal 50 PG® (phosphatidylcholine, propylene glycol, mono- and di-glycerides, ethanol, soy fatty acids, and ascorbyl palmitate) and polysorbate 80. RAPAMUNE Oral Solution contains 1.5%-2.5% ethanol.

The inactive ingredients in RAPAMUNE Tablets include: sucrose, lactose, polyethylene glycol 8000, calcium sulfate, microcrystalline cellulose, pharmaceutical glaze, talc, titanium dioxide, magnesium stearate, povidone, poloxamer 188, polyethylene glycol 20,000, glyceryl monooleate, carnauba wax, *dl*-alpha tocopherol, and other ingredients. The 0.5 mg and 2 mg dosage strengths also contain yellow iron (ferric) oxide and brown iron (ferric) oxide.

7.4.2 **Form**

Sirolimus is available for oral administration as an oral solution and tablets. Oral solution contains 60mg per 60ml in an amber glass bottle. Tablets contain 0.5 mg, 1 mg, or 2 mg. Injection is available as solution containing 5mg in 1 ml.

7.4.3 **Storage and Stability**

Solution bottles should be stored protected from light and refrigerated at 2°C to 8°C (36°F to 46°F). Once the bottle is opened, the contents should be used within one month. If necessary, the patient may store the bottles at room temperatures up to 25°C (77°F) for a short period of time (e.g., not more than 15 days for the bottles).

An amber syringe and cap are provided for dosing, and the product may be kept in the syringe for a maximum of 24 hours at room temperatures up to 25°C (77°F) or refrigerated at 2°C to 8°C (36°F to 46°F). The syringe should be discarded after one use. After dilution, the preparation should be used immediately.

Solution provided in bottles may develop a slight haze when refrigerated. If such a haze occurs, allow the product to stand at room temperature and shake gently until the haze disappears. The presence of this haze does not affect the quality of the product.

Tablets should be stored at 20° to 25°C [USP Controlled Room Temperature] (68° to 77°F). Use cartons to protect blister cards and strips from light. Dispense in a tight, light resistant container as defined in the USP.

7.4.4 **Compatibility**

Do not mix sirolimus solution with anything other than water or orange juice. Do not crush, split, or chew tablets. May be taken with or without food, but should be taken consistently with regard to food (always on an empty stomach or always with food).

7.5 Recombinant Human Granulocyte Colony Stimulating Factor

7.5.1 **Description**

Neupogen or TBO-Filgrastim (Granix) is a human granulocyte colony-stimulating factor (G-CSF), produced by recombinant DNA technology. Either may be used in this study.

Colony-stimulating factors are glycoproteins which act on hematopoietic cells by binding to specific cell surface receptors and stimulating proliferation, differentiation commitment, and some end-cell functional activation. Endogenous G-CSF is a lineage specific colony-stimulating factor which is produced by monocytes, fibroblasts, and endothelial cells. G-CSF regulates the production of

neutrophils within the bone marrow and affects neutrophil progenitor proliferation, differentiation, and selected end-cell functional activation (including enhanced phagocytic ability, priming of the cellular metabolism associated with respiratory burst, antibody dependent killing, and the increased expression of some functions associated with cell surface antigens. G-CSF is not species specific and has been shown to have minimal direct in vivo or in vitro effects on the production of hematopoietic cell types other than the neutrophil lineage.

7.5.2 **Form**

TBO-Filgrastim (Granix): A sterile, clear, colorless, preservative-free liquid for parenteral administration available as single use prefilled syringes only. The single use prefilled syringes contain either 300 mcg or 480 mcg TBO-Filgrastim (Granix) at a fill volume of 0.5 mL or 0.8 mL, respectively.

Filgrastim (Neupogen): A sterile, clear, colorless, preservative-free liquid for parenteral administration available as single use vials and prefilled syringes. The single use vials contain either 300 mcg or 480 mcg Filgrastim at a fill volume of 1.0 mL or 1.6 mL, respectively. The single use prefilled syringes contain either 300 mcg or 480 mcg Filgrastim at a fill volume of 0.5 mL or 0.8 mL, respectively.

7.5.3 **Storage and Stability**

Vials and syringes should be stored at 2° to 8°C (36° to 46°F). Avoid shaking. May be administered by I.V. bolus, or a short infusion over 15-30 minutes in D_5W, or by continuous I.V. infusion. Filgrastim diluted with D_5W for I.V. infusion (5-15 mcg/mL) is stable for 7 days at 2°C to 8°C (36°F to 46°F), however, should be used within 24 hours due to the possibility for bacterial contamination.

7.5.4 **Compatibility**

Stable in D5W

7.5.5 **Handling**

Use appropriate precautions for handling and disposal

7.5.6 **Administration**

G-CSF will be administered subcutaneously or intravenously per institutional guidelines

8. CORRELATIVE/SPECIAL STUDIES

8.1 Functional Reconstitution of T Cell Immunity

It is possible that elimination of ATG from the conditioning regiment may selectively affect certain T cell subsets and alter T cell polarization. For this reason, we will evaluate Th1 (producing IFN- , Th2 (IL-4) and Th17 (producing IL-17) cells by intracellular staining and flow cytometry by assessing expression of each of these cytokines on CD4+ cells. To assess and confirm polarization, we will also examine the expression of lineage-specific transcription factors T-bet for Th1 cells, GATA3 for Th2 cells, Foxp3 and Runx1 for Treg, RORC and Runx1 for Th17 cells. We will also examine the effect of ATG elimination from the conditioning regiment on functional immune reconstitution, by assessing GvHD, GvL and pathogen-specific response in UCBT recipients.
We will assess the following endpoints:

1. Thymic regeneration by assessing signal joint TCR excision circle (TREC) DNA.
2. Quantitative reconstitution of pathogen-specific effectors (specific for CMV, EBV and aspergillus) by using pathogen/MHC-specific pentamers.
3. Qualitative immune reconstitution by assessing function of pathogen-specific effectors by ELISpot.
4. Quantitative reconstitution of leukemia-specific effectors by using WT1/MHC-specific pentamers**.**
5. Induction of allo-specific anergy vs. GvHD.

We will use patients' T cells -that are of donor origin- to stimulate *in vitro* with DC that we have generated from each patient prior to transplantation (specific allostimulator DC) or with third party allo-stimulator DC and frequency of HTLp and or CTLp will be examined by methods previously established in our laboratory to determine whether such frequencies are above or below the level predictive for clinical GvHD. All the data will be compared to the relevant results generated from patients on a previous clinical trial of UCBT treated with the same conditioning regiment, which in addition contained ATG. These studies will be done in the laboratory of Dr. Vicki Boussiotis.

15 ml of blood in yellow top tubes will be collected at the following time points as outlined In Table 2:

1. Pre Transplant
2. Days 30 +/- 3 days, 60 +/- 3 days, 100 +/- 7 days post transplant
3. Months 6, 9, 12, and 24 post transplant, all +/- 14 days

The tubes will be transported by courier from MGH and hand picked up at DFCI and BIDMC. (Please see Appendix 2)

A secondary endpoint in this protocol is to monitor immune reconstitution after stem cell transplantation and to compare results with similar patients treated in previous protocols in which ATG was used in the conditioning regimen. Although ATG is

administered before infusion of umbilical cord blood stem cells, clearance of ATG from the recipient is delayed and is known to affect donor cells. Preparative regimens that do not include ATG may therefore lead to enhanced reconstitution of donor stem cells. This will therefore be monitored in the current study. These studies will be performed under the direction of Dr. Jerome Ritz. (Please see Appendix 3)

Immune reconstitution after stem cell transplantation will be monitored by analysis of peripheral blood samples obtained at time points outlined in Table 2. At each time point, numbers of circulating T, B and NK cells will be quantified using a panel of directly conjugated monoclonal antibodies (CD3, CD4, CD8, CD19/CD20 and CD56). Samples will be analyzed by flow cytometry and results compared to previous studies using similar methods in patients who received ATG containing transplant conditioning regimens. Since CD 4 has correlated with transplant outcome in our earlier studies, CD 4 count will be a main parameter to follow. In addition to phenotypic studies outlined above, samples of peripheral blood mononuclear cells and plasma will be cryopreserved at each time point. These samples can be used for more detailed phenotypic and functional studies as well as measurements of plasma cytokines if indicated.

8.2 Optional Thrombopoietin Levels

Patients may elect to participate in the thrombopoietin (TPO) level portion of the study.

To understand the changes in TPO levels after double cord blood transplant, we will measure TPO levels after transplantion. TPO levels will be determined with the assistance of Dr. David Kuter and Dr. Robert Makar.

TPO levels will be measured:
1. Pre conditioning
2. Day 0
3. Day 14, Day 30 and Day 60+/-3 days
4. Day 100 +/- 7 days
5. Month 6, Month 9+/-14 days
6. Month 12, 24+/-14 days

Another 4 TPO samples will be obtained in association with platelet transfusions occurring after transplantation. With each eligible platelet transfusion, BOTH a CBC and a sample for TPO assessment will be obtained:

1. Immediately prior to the platelet transfusion (TPO level only)
2. 30-60 minutes after the completion of the transfusion
3. 6 hours after the completion of the transfusion (+/- 60 min)
4. 24 hours after the completion of the transfusion (+/- 60 min)

TPO samples will obtained in -at least 1 and up to 3 eligible platelet transfusions in each patient.

Due to the requirement for sample processing at the time of collection, only platelet transfusions administered on Mondays, Tuesdays, Wednesdays or Thursdays will be considered. Transfusions should be administered prior to 10 AM if possible to allow for collection of the TPO level specimen during normal business hours.

In the event that 2 transfusions are administered within the same 24 hour period, only the first transfusion is eligible for TPO measurements.

Since these assessments are entirely voluntary and optional, there will be no protocol deviations or violations for missed samples.

TPO specimens will be collected in large red top tubes and must be refrigerated at 4C within 8 hours of collection.

Dr. Kuter has an MTA with Quest Nichols Institute in which they have agreed to provide TPO results on clinical specimens. Dr. Makar will oversee this process.

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Specimens are processed as follows (Please see Appendix 1):

1. Clotted blood from a large red top tube is spun for 15 minutes at 1500 RCF to separate the serum from the clot. The red top tubes may be stored at 4C for up to 24 hours and then batched for processing.
2. The serum is aliquoted into tubes labeled with the date and a numerical identifier. For each red top tube, prepare a minimum of 2 samples each containing a minimum of 1 ml of serum.
3. If the clot is insufficiently packed down to allow easy collection of both serum samples, spin the red top tube again at 1500 RCF for 15 minutes.
4. The date and numerical identifier of each serum sample prepared are entered into a database.
5. Serum samples are then frozen at -20C or colder pending shipment to Quest Nichols Institute. Use only a **frost-free** freezer for storing these specimens.
6. TPO samples that have been collected and stored may be shipped for testing approximately once every month.
7. A Quest Cambridge courier picks up specimens from the Core Lab at MGH and from the DFCI and BIDMC.

8.3 Pharmacokinetic Studies:
N/A

8.4 Pharmacodynamic Studies:
N/A

9. STUDY CALENDAR

9.1 Pre Transplant Evaluations

All required tests must be performed within 42 days of admission for transplantation.

Clinical Evaluations

1. 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. A complete physical examination.
3. Chest and other radiographs as clinically indicated. Patients in whom CT or PET-CT scan has been the predominant staging modality prior to transplantation must have these repeated.
4. Marrow aspiration and biopsy for staging, cytogenetics, and flow cytometry in patients in whom this test has previously been informative. If available to have procedure on site, patients undergoing bone marrow aspirate and biopsy may have a bone marrow aspirate sample sent to the Pasquarello lab for future Immunologic Reconstitution studies.
5. EKG
6. Dental consult and evaluation of status of teeth and gums (may be done within 6 months of study entry)
7. Pulmonary function tests (excluding patients with a DLCO of < 50%).
8. Echocardiogram or MUGA to assess ejection fraction.
9. .

Laboratory Evaluations

1. HLA typing of patient and available family members. The patient will be typed at HLA A, B, C and DRβ1 by high resolution molecular typing. HLA typing may be performed more than 42 days prior to admission.
2. HLA antibody testing. HLA antibody testing may be performed more than 42 days prior to admission.
3. ABO and Rh typing
4. CBC with differential
5. Comprehensive chemistry profile, including measures of hepatic and renal function.
6. Hepatitis B surface antigen, HCV, HSV, CMV, EBV, HHV-6, HIV and HTLV-I antibody determinations per institutional guidelines.
7. Genotyping samples sent to the tissue typing laboratory for future chimerism analysis. A sample from the cord units prior to transplantation will be processed similarly.
8. Blood sample sent for Immunologic Reconstitution assays (Dr. Ritz).
9. Blood sample sent for T cell subsets and polarization (Dr. Boussiotis)
10. Blood sample sent for thrombopoietin level if participating.
11. HHV6 by DNA will be done prior to transplant.
12. If possible, if a cord unit segment is available, the cord units will be tested for HHV-6 by PCR.

9.2 Post Transplant Evaluation

Standard post-transplantation testing, including complete blood counts, serum chemistries, measurements of immunosuppressant levels will be performed per institutional guidelines and consistent with clinical good practice.

Chimerism studies will be performed by short tandem repeats and/or flow cytometry according to local laboratory practices. When feasible, fractional chimerism (total, myeloid, lymphoid) will be performed from peripheral blood sample

Table 2: Required Data

	Pre-transplant	Weekly to Day 100	Day +14, +30 and +60[1]	Day +30, and +60	Day +100[2]	Monthly from Day +100 to 1 year[3]	Month 6 and 9[3]	Months 12 and 24[3]
CBC with diff[9]	X	X	X		X		X	X
Comprehensive chemistry with renal and liver functions	X	X	X		X		X	X
HLA typing	X							
Chimerism	X		X		X		X	X
ABO/Rh	X				X			
Infectious disease markers including HHV-6, EBV and HSV	X							
T-cell subsets and polarization[4]	X			X	X		X	X
Optional thrombopoeitin levels[5]	X		X		X		X	X
Immune reconstitution/blood banking[6]	X		X		X		X	X
Bone marrow biopsy and aspirate[7]	X				X			X
Radiologic tumor staging[8]	X				X		X	X
History and physical,	X				X		X	X
EKG	X							
MUGA or ECHO	X							
PFTS	X X							
Dental exam	X							
Chest x-ray	X							
Tacrolimus and sirolimus levels		X			X		X	X
CMV,EBV and HHv-6 assays[10]	X	X			X	X	X	X
GVHD and toxicity assessment[11]		X		x	X		X	X
Cord Blood Unit for HHV-6[-12]								

[1] +/- 3 days

[2]+/- 7 days

[3]+/- 14 days

[4]15 ml of blood is required at each time point, please see appendix 2

[5]Please see appendix 1

[6]Peripheral blood will be banked and analyzed for reconstitution of immune cells after transplantation. See Appendix 3

[7]A complete marrow assessment will include an aspirate for morphology, flow cytometry, cytogenetics (with FISH where indicated), chimerism assays and tissue banking. A biopsy should be performed and the marrow aspirate sample for tissue banking is sent to the Pasquarello lab

[8]CT or PET-CT should be employed, consistent with the most informative test prior to transplantation. Not required for patients with marrow-only disease (i.e. AML, MDS, CML)

[9]Differentials are at the discretion of the treating MD

[10] Human herpes virus 6 (HHV-6) by PCR HHV-6 by PCR should be done prior to transplant.

[11] Documentation of toxicity assessments are only required weekly for the first month, then on Day 60+, 100+ +/- 7 days, Month 6, 9, 12, 24+/- 14 days

[12] If possible, if a cord unit segment is available, the cord units will be tested for HHV-6 by PCR.

10. MEASUREMENT OF EFFECT

10.1 Engraftment

10.1.1 Time to Neutrophil Engraftment

The time to neutrophil engraftment is defined as the first of 3 consecutive days of absolute neutrophil count > 500.

Primary graft failure is defined as the failure to achieve an ANC >500/ μL by day 42, in the absence of relapse. Secondary graft failure is defined as initial neutrophil engraftment followed by subsequent decline in neutrophil counts >500/ μL.

10.1.2 Time to Platelet Engraftment

Platelet engraftment is defined as a platelet count ≥ 20,000/μL for three consecutive measurements over three or more days. The first of the three days will be designated the day of platelet engraftment. Subjects must not have had platelet transfusions during the preceding 3 days or in the following 7 days after the day of engraftment, unless the platelet transfusion is being given specifically to achieve a platelet threshold to allow an elective invasive procedure, such as a central catheter removal. The time to a platelet count ≥ 100,000/μL will be collected as well.

10.2 Incidence of GVHD

10.2.1 Incidence of Acute GVHD

Acute GVHD will be scored according to the Consensus Criteria (Tables 3, 4). The incidence of Gr. II-IV and grade III-IV acute GVHD will be noted

Table 3: Organ Staging of GVHD

Stage	Skin	Liver	Gut

0	No rash due to GVHD	Bilirubin < 2 mg/dL	< 500 mL diarrhea per day
1	Maculopapular rash < 25% of body surface[a]	Bilirubin 2-3 mg/dL[b]	500 to 999 mL diarrhea per day[c] or persistent nausea with histologic evidence of GVHD in stomach/ duodenum
2	Maculopapular rash 25-50% of body surface[a]	Bilirubin 3.1-6 mg/dL[b]	1,000 to 1,499 mL diarrhea per day
3	Maculopapular rash > 50% of body surface[a]	Bilirubin 6.1-15 mg/dL[b]	1,500 or more mL diarrhea per day[c]
4	Generalized erythroderma with bullous formation	Bilirubin > 15 mg/dL[b]	Severe abdominal pain with or without ileus

[a] Use "Rule of Nines" to determine extent of rash.
[b] Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubin has been documented.
[c] Downgrade one stage if an additional cause of diarrhea has been documented.

Table 4: Overall Clinical Grading of Severity of Acute GVHD

Grade	Degree of Organ Involvement
0	No Stage 1-4 of any organ
I	Stage 1-2 rash and no liver or gut involvement
II	Stage 3 rash, or Stage 1 liver involvement, or Stage 1 gut involvement
III	None to Stage 3 skin rash with Stage 2-3 liver involvement, or Stage 2-4 gut involvement
IV	Stage 4 skin rash, or Stage 4 liver involvement

10.2.2 Chronic GVHD

Chronic GVHD is scored according to the NIH consensus criteria.[45] The first day of chronic GVHD onset will be used to calculate cumulative incidence curves.

10.3 Other Response Parameters

10.3.1 Treatment Related Mortality

All deaths in the absence of relapse of the primary malignancy will be considered treatment related mortality. The cumulative incidence of treatment related mortality at 30 and 100 days will be measured.

10.3.2 Immunologic Reconstitution

Blood and marrow samples will be obtained and stored in the Pasquarello tissue bank for future immunologic assays, under the supervision of Dr. Jerry Ritz. Immune reconstitution after stem cell transplantation will be monitored by analysis of peripheral blood samples obtained at time points outlined in Table 2. At each time point, numbers of circulating T, B and NK cells will be quantified using a panel of directly conjugated monoclonal antibodies (CD3, CD4, CD8, CD19/CD20 and CD56). Samples will be analyzed by flow cytometry and results compared to previous studies using similar methods in patients who received ATG containing transplant conditioning regimens. Since CD4 count has been shown to have prognostic significance after reduced intensity cord blood transplant, CD4 count will be the main immune reconstitution endpoint for this analysis. [20]

10.3.3 Relapse-Free and Overall Survival

Both overall survival and relapse-free survival will be assessed one and two years from the date of stem cell infusion. Patients alive at the time of last observation will be censored. Testing for recurrent malignancy in the blood, marrow or other sites will be used to assess relapse after transplantation. Relapse is defined by either morphological or cytogenetic evidence of the original malignancy consistent with pre-transplant features. Minimal residual disease is defined by the sole evidence of malignant cells by flow cytometry, or fluorescent in situ hybridization (FISH), or Southern blot, or Western blot, or polymerase chain reaction (PCR), or other techniques, in absence of morphological or cytogenetic evidence of disease in blood or marrow. Since the frequency of testing for minimal residual disease is highly variable, and the sensitivity is highly variable among laboratory techniques, evidence of minimal residual disease will not be sufficient to meet the definition of relapse in the context of this study.

10.3.4 FractionalChimerism of Cord Blood Units

Chimerism of transplanted cord blood units will be measured according to the SOPs of the treating institution. At all assessments, where technically feasible, unfractionated, myeloid and lymphoid chimerism will be measured from peripheral blood specimens. Unfractionated chimerism will be measured from bone marrow specimen.

A cord blood unit will be considered to be *dominant* if it contributes ≥ 50% of hematopoiesis as measured by chimerism at that time point.

10.3.5 Second Malignancies

Patients will be followed for the development of second cancers, including lymphoproliferative disorder and myelodysplasia/myeloproliferative disorder. Second malignancies will be determined to be of either donor or recipient origin by cytogenetics or chimerism assays where possible.

10.4 Evaluation of Response

All participants included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each participant

should be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database. World Health Organization (WHO) criteria will be used to assess response.

11. ADVERSE EVENT REPORTING REQUIREMENTS

11.1 Definitions

11.1.1 Adverse Event (AE)

An adverse event (AE) is any undesirable sign, symptom or medical condition or experience that develops or worsens in severity after starting the first dose of study treatment or any procedure specified in the protocol, even if the event is not considered to be related to the study.

Abnormal laboratory values or diagnostic test results constitute adverse events only if they induce clinical signs or symptoms or require treatment or further diagnostic tests.

11.1.2 Serious adverse event (SAE)

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more sever form.
- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person’s ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical

intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

11.1.3 Expectedness

Adverse events can be 'Expected' or 'Unexpected.'

11.1.3.1 Expected adverse event

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For the purposes of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk.

Refer to Section 6.1 for a listing of expected adverse events associated with the study agent(s).

11.1.3.2 Unexpected adverse event

For the purposes of this study, an adverse event is considered unexpected when it varies in nature, intensity or frequency from information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

11.1.4 Attribution

Attribution is the relationship between an adverse event or serious adverse event and the study treatment. Attribution will be assigned as follows:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.

- Possible – The AE may be related to the study treatment.
- Unlikely - The AE is doubtfully related to the study treatment.
- Unrelated - The AE is clearly NOT related to the study treatment.

11.2 Procedures for AE and SAE Recording and Reporting

Participating investigators will assess the occurrence of AEs and SAEs at all participant evaluation time points during the study.

All AEs and SAEs whether reported by the participant, discovered during questioning, directly observed, or detected by physical examination, laboratory test or other means, will be recorded in the participant's medical record and on the appropriate study-specific case report forms.

The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website at:

http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

11.3 Reporting Requirements

For multi-site trials where a DF/HCC investigator is serving as the principal investigator, each participating investigator is required to abide by the reporting requirements set by the DF/HCC. The study must be conducted in compliance with FDA regulations, local safety reporting requirements, and reporting requirements of the principal investigator.

Each investigative site will be responsible to report SAEs that occur at that institution to their respective IRB. It is the responsibility of each participating investigator to report serious adverse events to the study sponsor and/or others as described below.

11.4 Reporting to the Study Sponsor

The Study Team is also responsible for providing Amgen a semi-annual report of any reported SAEs and AEs related to these events of interest:

- Thrombocytosis
- Renal Impairment
- Off-label use
- Medication Error
- Leukocytosis and Anemia
- Immunogenicity
- Hematopoietic Malignancy/MDS
- Haemorrhages

- Bone Marrow Reticulin/Bone Marrow Fibrosis

REPORTING	FREQUENCY
SUSARs	Within one business day of IRB submission
SADRs	Within One month of the event
SAEs	Semi-Annual Report of individual events
SAE/AE Events of Interest	Semi-Annual Report
Pregnancy and/or Lactation Exposure	Within 15 days

11.4.1 Serious Adverse Event Reporting

All serious adverse events that occur after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment must be reported to the DF/HCC Overall Principal Investigator on the local institutional SAE form. This includes events meeting the criteria outlined in Section 11.1.2, as well as the following:

- Grade 2 (moderate) and Grade 3 (severe) Events – Only events that are unexpected and possibly, probably or definitely related/associated with the intervention. Expected Grade 3 events will NOT be routinely reported.

- All Grade 4 (life-threatening or disabling) Events – Expected and Unexpected events will be reported, with the exception of those events that are routinely expected after transplantation with this conditioning regimen. Examples of these events include: neutropenia with fever, thrombocytopenia with minor bleeding, mucositis with inability to eat, fatigue/malaise, etc.

All Grade 5 (fatal) Events – When the participant is enrolled and actively participating in the trial OR when the event occurs within 30 days of the last study intervention. Deaths related to relapse or progression of the original malignancy are NOT reported as adverse events.

Note: If the participant is in long term follow up, report the death at the time of continuing review.

Participating investigators must report each serious adverse event to the DF/HCC Overall Principal Investigator within 24 hours of learning of the occurrence. In the event that the participating investigator does not become aware of the serious adverse event immediately (e.g., participant sought treatment elsewhere), the

participating investigator is to report the event within 24 hours after learning of it and document the time of his or her first awareness of the adverse event. Report serious adverse events by telephone, email or facsimile to:

Zachariah DeFilipp, MD
[redacted]
Email: zdefilipp@mgh.harvard.edu
[redacted]

Within the following 24-48 hours, the participating investigator must provide follow-up information on the serious adverse event. Follow-up information should describe whether the event has resolved or continues, if and how the event was treated, and whether the participant will continue or discontinue study participation.

11.4.2 Non-Serious Adverse Event Reporting

Non-serious adverse events will be reported to the DF/HCC Overall Principal Investigator on the toxicity Case Report Forms.

11.5 Reporting to the Institutional Review Board (IRB)

Investigative sites within DF/HCC will report all serious adverse events directly to the DFCI Office for Human Research Studies (OHRS).

11.6 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any subject safety reports or sentinel events that require reporting according to institutional policy.

11.7 Reporting to the FDA, NIH Office of Biotechnology Activity (OBA), Institutional Biosafety Committee (IBC)

N/A

11.8 Monitoring of Adverse Events and Period of Observation: All adverse events, both serious and non-serious, and deaths that are encountered from initiation of study intervention, throughout the study, and within 30 days of the last study intervention should be followed to their resolution, or until the participating investigator assesses them as stable, or the participating investigator determines the event to be irreversible, or the participant is lost to follow-up. The presence and resolution of AEs and SAEs (with dates) should be documented on the appropriate case report form and recorded in the participant's medical record to facilitate source data verification.

For some SAEs, the study sponsor or designee may follow-up by telephone, fax, and/or monitoring visit to obtain additional case details deemed necessary to appropriately evaluate the SAE report (e.g., hospital discharge summary, consultant report, or autopsy report).

Participants should be instructed to report any serious post-study event(s) that might reasonably be related to participation in this study. Participating investigators should notify the DF/HCC Overall Principal Investigator and their respective IRB of any unanticipated death or adverse event occurring after a participant has discontinued or terminated study participation that may reasonably be related to the study

12. DATA AND SAFETY MONITORING

12.1 Data Reporting

12.1.1 Method

The QACT will , manage, and monitor data for this study.

12.1.2 Data Submission

The schedule for completion and submission of case report forms (paper or electronic) to the QACT is as follows:

Form	**Submission Timeline**
Eligibility Checklist	Complete prior to registration with QACT
On Study Form	Within 14 days of registration
Baseline Assessment Form	Within 14 days of registration
Treatment Form	Within 10 days of transplant
Adverse Event Report Form	Within 10 days of the event
Response Assessment Form	Within 10 days of the completion of response evaluation
Off Treatment/Off Study Form	Within 14 days of completing treatment or being taken off study for any reason
Follow up/Survival Form	Within 14 days of the protocol defined follow up visit date or call

12.2 Safety Meetings

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this trial. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Principal Investigator and study team.

The DSMC will meet quarterly and/or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days for Phase I or II protocols; for gene transfer protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Monitoring

Involvement in this study as a participating investigator implies acceptance of potential audits or inspections, including source data verification, by representatives designated by the DF/HCC Overall Principal Investigator (or Protocol Chair) or DF/HCC. The purpose of these audits or inspections is to examine study-related activities and documents to determine whether these activities were conducted and data were recorded, analyzed, and accurately reported in accordance with the protocol, institutional policy, Good Clinical Practice (GCP), and any applicable regulatory requirements.

All data will be monitored for timeliness of submission, completeness, and adherence to protocol requirements. Monitoring will begin at the time of participant registration and will continue during protocol performance and completion.

13. REGULATORY CONSIDERATIONS

13.1 Protocol Review and Amendments

This protocol, the proposed informed consent and all forms of participant information related to the study (e.g., advertisements used to recruit participants) and any other necessary documents must be submitted, reviewed and approved by a properly constituted IRB governing each study location.

Any changes made to the protocol must be submitted as amendments and must be approved by the IRB prior to implementation. Any changes in study conduct must be reported to the IRB. The DF/HCC Overall Principal Investigator (or Protocol Chair) will disseminate protocol amendment information to all participating investigators.

All decisions of the IRB concerning the conduct of the study must be made in writing.

13.2 Informed Consent

All participants must be provided a consent form describing this study and providing sufficient information for participants to make an informed decision about their participation in this study. The formal consent of a participant, using the IRB approved consent form, must be obtained before the participant is involved in any study-related procedure. The consent form must be signed and dated by the participant or the participant's legally authorized representative, and by the person obtaining the consent. The participant must be given a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

13.3 Ethics and Good Clinical Practice (GCP)

This study is to be conducted according to the following considerations, which represent good and sound research practice:

- E6 Good Clinical Practice: Consolidated Guidance
  www.fda.gov/downloads/RegulatoryInformation/Guidances/UCM129515.pdf

- US Code of Federal Regulations (CFR) governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki

    - Title 21 Part 11 – Electronic Records; Electronic Signatures
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr11_02.html

    - Title 21 Part 50 – Protection of Human Subjects
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr50_02.html

    - Title 21 Part 54 – Financial Disclosure by Clinical Investigators
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr54_02.html

    - Title 21 Part 56 – Institutional Review Boards
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr56_02.html

    - Title 21 Part 312 – Investigational New Drug Application
      www.access.gpo.gov/nara/cfr/waisidx_02/21cfr312_02.html

- State laws

- DF/HCC research policies and procedures
  http://www.dfhcc.harvard.edu/clinical-research-support/clinical-research-unit-cru/policies-and-procedures/

It is understood that deviations from the protocol should be avoided, except when necessary to eliminate an immediate hazard to a research participant. In such case, the deviation must be reported to the IRB according to the local reporting policy.

13.4 Study Documentation

The investigator must prepare and maintain adequate and accurate case histories designed to record all observations and other data pertinent to the study for each research participant. This information enables the study to be fully documented and the study data to be subsequently verified.

Original source documents supporting entries in the case report forms include but are not limited to hospital records, clinical charts, laboratory and pharmacy records, recorded data from automated instruments, microfiches, photographic negatives, microfilm or magnetic media, and/or x-rays.

13.5 Records Retention

All study-related documents must be retained for the maximum period required by applicable federal regulations and guidelines or institutional policies.

13.6 Multi-Center Guidelines

N/A

13.7 Cooperative Research and Development Agreement N/A

14. STATISTICAL CONSIDERATIONS

This is a Phase II study designed to determine the significant viral infection rate at one year with the use of fludarabine, melphalan, and low dose radiation (LD TBI) as conditioning regimen for double umbilical cord blood transplantation (DUCBT). Clinically significant viral infection is defined as a viral infection that requires medical intervention..We will compare the incidence of viral infection requiring intervention to our historical control population of DUCBT with fludarabine (Flu), melphalan (Mel), and antithymocyte globulin (ATG). Viral infection requiring intervention is defined as follows:

1. CMV reactivation or infection requiring treatment such as ganciclovir, valganciclovir, foscarnet, or lymphocyte preparations (for example, third party Cytotoxic T lymphocytes)
2. EBV reactivation requiring rituximab, chemotherapy, or lymphocyte preparations.
3. HHV-6 reactivation requiring Foscarnet or lymphocyte preparations.
4. Adenovirus infection requiring treatment, such as cidofovir or lymphocyte preparations.
5. Herpes Zoster infection requiring treatment, such as high dose acyclovir.

Each patient can only be counted once toward the primary endpoint. In the event that a single patient has multiple viral infections, the time to the first infection will be counted for reporting purposes. The type of infection will also be recorded. All viral infections will be recorded, but only the above infections will count towards the primary endpoint.

14.1 Study Design/Endpoints

14.1.1 Primary Endpoint

The primary endpoint is significant viral infection by one year of transplant. Several transplant centers reported a reduction in viral infection without ATG. Although the overall viral infection rate was not reported, the Minnesota group [23] reported that the incidence of EBV related complications was reduced from 21% with ATG to 2% without ATG. The Memorial Sloan-Kettering group[44] has also reported a reduced viral infection rate (30% between day 31 and 60) without ATG in 72 patients who underwent myeloablative and non-myeloablative DUCBT. In our previous DUCBT study with Flu/Mel/ATG (DFCI#05154), the one year significant viral infection rate was 53%. Based on this information and extrapolating the Minnesota and Memorial Sloan-Kettering experience, if the one year significant viral infection rate is reduced to 33% or lower, the proposed conditioning regimen will be regarded as promising for reducing viral infections.

The study for the primary endpoint is a one-stage design with the accrual goal of 31 eligible patients..

With 31 patients, if 12 or fewer patients experience significant viral infection requiring intervention in the first year of transplant, the proposed conditioning regimen will be considered promising. With this design, the probability of concluding this regimen promising is 0.81 if the true but unknown viral infection rate is 33% and 0.08 if the true rate is 53%. This decision rule is calculated using an exact binomial distribution. Table 5 presents the operating characteristics of this design.[46-51]

Table 5 Operating Characteristics for the Primary Endpoint

	True but Unknown One Year Viral Infection Rate				
	53%	48%	43%	38%	33%
Prob (≤ 12 pts with viral infections within one year in 31 pts.)	0.08	0.20	0.38	0.61	0.81

14.1.2. Interim Analysis

Several transplant groups reported a reduced viral infection rate without ATG. However, no detailed information has been provided as to the exact magnitude of the reduction without ATG compared to with ATG in the nonmyeloablative setting. We therefore will conduct an interim analysis to monitor the efficacy of the proposed conditioning regimen on the viral infection. The interim analysis will take place when the first 13 patients receive the conditioning regimen, undergo DUCBT, and followed for one year. Of these 13 patients, if 7 or more patients experience a clinically significant viral infection within one year of transplant, we will provide the results of the interim analysis to the Data and Safety Monitoring Committee (DSMC) as a guideline for their decision on whether to proceed with the study. With this design, the probability of consulting with DSMC is 0.1 if the true but unknown one year viral infection rate is 33%, but 0.59 if the rate is 53%.

In addition, when these 13 patients are followed for 42 days after the DUCBT, we will also evaluate the neutrophil engraftment. Although we do not anticipate that substituting LD TBI for ATG will delay the neutrophil engraftment (see Section 10 for the definition), we will monitor the neutrophil engraftment in parallel. In the previous DUCBT at DF/HCC, 85- 90% of patients achieved neutrophil engraftment by day 42 post transplant. Based on this information, if 9 or fewer patients achieve neutrophil engraftment by day 42 in the first 13 patients, we will consult with the DSMC as to whether the accrual should stop. With this design, the probability of consulting with the DSMC is 0.03 if the true but unknown rate of day 42 neutrophil engraftment is 90%, 0.12 if the rate is 85%, and 0.72 if the rate is 65%.

Results of Interim Analysis

As of February 12, 2013, 16 patients were enrolled, among whom 14 patients were transplanted. Of these 14, data for the primary endpoint was available for the first 13 patients, and these 13 patients are included in the interim analysis. Of these 13, 8 patients experienced clinically significant infections (5 HHV-6, 2 CMV, 1 both CMV and HHV-6), 1 patient experienced graft failure, 1 patient experienced grade III-IV acute GVHD and 1 patient died of TRM within 100 days of transplantation. Since the total number of infections exceeds the threshold of 7, as specified in the original design, the accrual has been temporarily suspended and the results of the interim analysis discussed with the DSMC.

On February 27, 2013, the study team met to review the data and to discuss the future direction and proposed the following amendments. 1) mandatory antiviral prophylaxis with Famvir 500 tid or Valtrex 1 gram daily (use IV Acyclovir 5mg/kg every 8 hours if patients cannot take p.o.); 2) limiting eligibility to patients age 65 and younger; and 3) conducting an interim analysis after 10 patients are enrolled and transplanted.

Stopping rules after study re-open

HHV-6 encephalitis
Due to the unexpectedly high incidence of HHV-6 in the first 13 patients, the incidence of HHV-6 encephalitis will be monitored closely. If in the 10 subsequently enrolled and transplanted patients that includes one patient who were transplanted but not included in the interim analysis, 3 or more patients experience HHV-6 encephalitis, the study will be terminated early. With this design, the probability of terminating the study early is 0.62 if the true but unknown rate of HHV-6 encephalitis is 30%, but 0.07 if the true rate is 10%.

Clinically Significant Viral infection
The primary endpoint of clinically significant viral infection will also be monitored closely. If in the 10 patients, 5 or more patients experience any of clinically significant infections of CMV, EBV, HHV-6, Adenovirus or Herpes Zoster, the study will be terminated early. Based on this decision rule, the probability of terminating the study is 0.69 if the true but unknown infection rate is 53%, but 0.21 if the rate is 33% and 0.12 if the rate is 28%.

Primary Graft failure
The primary graft failure will be continuously monitored. If 3 or more graft failures are observed in the 10 subsequently enrolled patients, the study will be terminated early. With this design, the probability of terminating the study early is 0.07 if the true but unknown graft failure rate is 10%, but 0.74 if the rate is 35%.

Results of Second Interim Analysis:
As of October 14, 2014, 11 additional patients were transplanted after the study was re-opened. Among the first 10 patients who were subsequently enrolled, 5 patients experienced clinically significant infections (3 CMV, 1 CMV and Herpes Zoster, 1 Adenovirus and HHV-6), therefore the study met the early stopping rule which was specified in the amendment following the first interim analysis (≥5 clinically significant infections in 10 subsequently enrolled patients).

The accrual to the study was temporarily suspended and the study team met on December 16, 2014. After reviewing additional outcome data, the study team felt that although the overall targeted reduction in infection rates will not be reached, the outcome was good among the 10 patients who were subsequently enrolled since the study re-opened with a more restricted age eligibility of under 65: no 100 day TRM, 17% TRM and 83% survival at 6 months, respectively. The study team suggested to re-open the study and to complete the accrual so more data can be gathered within the group with age under 65 (7 more patients need to be accrued). Secondary endpoints of interest include the TPO levels, relapse rate, and GVHD with this regimen.

14.2 Sample Size/Accrual Rate

The target accrual is 31 evaluable patients.

Based on our current practice, the accrual rate is expected to be approximately 13 patients per year. Thus, we anticipate that the accrual will complete in 2.5 years. Of note, the accrual will not be suspended during the interim analysis.

14.3 Analysis of Secondary Endpoints

In addition to the reduction in the significant viral infection rate, we anticipate that the second cancer rate that includes PTLD and the non-relapse mortality (NRM) rate will also be decreased, but relapse rate will be unchanged. Descriptive analysis will be performed for the incidence of second cancer, and competing risks data analysis will be performed for the incidence of NRM and relapse reflecting these two events as competing risks. Competing risks data analysis will also be performed for the cumulative incidence of (acute or chronic) GVHD accounting relapse or death without developing GVHD for a competing risk, and for the time to neutrophil and platelet engraftment accounting graft failure for a competing risk. For the overall survival and disease-free survival, standard survival analysis will be performed. For the analysis of TPO levels and immune reconstitution (T, B, NK cell numbers with emphasis on CD4 count), graphical assessment of levels over time as well as repeated measures analysis will be performed. In addition, correlation analysis between TPO levels and platelet counts and number of platelet transfusions will be performed, and repeated measurements of CD4 count will be assessed as a time dependent variable in proportional hazards model.

14.4 Reporting and Exclusions

14.4.1 **Evaluation of toxicity.**

Adverse events will be monitored from the time of admission for pre-transplant conditioning through 100 days from the time of stem cell infusion (day +100). See Section 11 for definitions of adverse events. Adverse events include:

- Death from any cause except in patients with active or recurrent malignancy after transplantation prior to day 100 after transplantation
- Severe Veno-occlusive disease of the liver
- Thrombotic Microangiopathy requiring renal replacement therapy
- Diffuse Alveolar Hemorrhage / Idiopathic Pneumonia Syndrome
- Other expected treatment-related toxicity related to transplantation (CTC Gr 4)
- Other unexpected treatment-related toxicity related to transplantation (CTC Gr 4)
- Infusional allergic reactions (CTC Gr. 3 – 4)
- Grade III-IV Acute GVHD

14.4.1.1 Severe VOD

Severe VOD is defined as the presence of VOD per the Baltimore Criteria with evidence of multi-organ failure as follows:

Baltimore Criteria for the Diagnosis of VOD:

- Jaundice (bilirubin ≥ 2 mg/dL) and at least 2 of the following 3 clinical findings:
    - Ascites
    - Weight gain ≥ 5% above baseline weight (defined as weight on the first day of conditioning or the weight on the date of admission)
    - Hepatomegaly (with pre-existing hepatomegaly there must be documentation by physical exam or imaging that liver size is increased over baseline)

14.4.1.2 Multi-organ failure, defined as the presence of one or both of the following:

- Renal dysfunction
    - Serum creatinine ≥ 3 x value on the date of admission or ≥ 3 x lowest value during conditioning prior to SCT (whichever is lowest)
    - Creatinine clearance or GFR ≤ 40% of admission value
    - Dialysis dependence
- Pulmonary Dysfunction
    - Documentation of oxygen saturation ≤ 90% on room air or requirement for oxygen supplementation/ventilator dependence. Dysfunction must be attributable to fluid overload or mechanical impingement from abdominal distention or hepatic enlargement and not to an infection

14.4.1.3 Thrombotic Microangiopathy

The diagnostic criteria for thrombotic microangiopathy include the concurrent occurrence of:

- Red cell fragmentation on a manual differential (2 + schistocytes) with a negative Coombs test
  and
- LDH > normal
  and either
- Renal dysfunction (doubling of serum creatinine or a decrease > 50% in the measured creatinine clearance)
  or
- Neurological dysfunction unexplained by another etiology

Thrombotic microangiopathy that requires renal replacement therapy (continuous or intermittent hemodialysis) will be required to define a severe adverse event.

15. PUBLICATION PLAN

The Principal Investigator and co investigators will plan to publish within one year of the end of data collection. The information may be published in abstract form prior to the end of data collection.

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17. APPENDICES

Appendix 1: Optional Thrombopoietin Levels

Delayed platelet engraftment is also an important contributor to the increased morbidity, poor quality of life, and cost after double cord blood transplant. Therefore, as an optional correlate, we will study thrombopoietin (TPO) levels after cord blood transplant, to determine if further investigation of TPO receptor agonists post cord blood transplant is warranted.

Patients may elect to participate in the thrombopoietin level portion of the study.

TPO levels will be determined with the assistance of Dr. David Kuter and Dr. Robert Makar.

TPO levels will be measured:
1. Pre conditioning
2. Day 0
3. Day 14+, Day 30+, Day 60+/- 3 days
4. Day 100 +/- 7 days
5. Month 6, Month 9+/-14 days
6. Month 12, Month 24+/-14 days

Another 4 TPO samples will be obtained in association with platelet transfusions occurring after transplantation. With each eligible platelet transfusion, BOTH a CBC and a sample for TPO assessment will be obtained:

1. Immediately prior to the platelet transfusion (TPO level only)
2 30-60 minutes after the completion of the transfusion
3. 6 hours after the completion of the transfusion (+/- 60 min)
4. 24 hours after the completion of the transfusion (+/- 60 min)

TPO samples will obtained in at least 1 and up to 3 eligible platelet transfusions in each patient.

Due to the requirement for sample processing at the time of collection, only platelet transfusions administered on Mondays, Tuesdays, Wednesdays or Thursdays will be considered. Transfusions should be administered prior to 10 AM if possible to allow for collection of the TPO level specimen during normal business hours.

In the event that 2 transfusions are administered within the same 24 hour period, only the first transfusion is eligible for TPO measurements.

Since these assessments are entirely voluntary and optional, there will be no protocol deviations or violations for missed samples..

TPO specimens will be collected in large red top tubes and must be refrigerated at 4C within 8 hours of collection.

Dr. Kuter has an MTA with Quest Nichols Institute in which they have agreed to provide TPO results on clinical specimens. Dr. Makar will oversee this process.

Specimens are processed as follows:

1. Clotted blood from a large red top tube is spun for 15 minutes at 1500 RCF to separate the serum from the clot. The red top tubes may be stored at 4C for up to 24 hours and then batched for processing.
2. The serum is aliquoted into tubes labeled with the date and a numerical identifier. For each red top tube, prepare a minimum of 2 samples each containing a minimum of 1 ml of serum.
3. If the clot is insufficiently packed down to allow easy collection of both serum samples, spin the red top tube again at 1500 RCF for 15 minutes.
4. The date and numerical identifier of each serum sample prepared are entered into a database.
5. Serum samples are then frozen at -20C or colder pending shipment to Quest Nichols Institute. Use only a **frost-free** freezer for storing these specimens.
6. TPO samples that have been collected and stored may be shipped for testing approximately once every month.
7. A Quest Cambridge courier picks up specimens from the Core Lab at MGH and from the DFCI and BIDMC.
8. The specimens are then shipped from the Cambridge Office to Nichols Institute.
9. To request sample pickup, the laboratory will email the following individuals 24 hours in advance:

10. In preparation for shipment, place the samples in a Nichols Institute bag provided by Quest. Affix to the bag a label with the following information:

11. Store the bag to be shipped at -20C (frost free) in a foam shipper. Include a shipping manifest listing the ID numbers of the serum specimens contained in the bag.

TPO Sample Collection Times*

Pre-conditioning
Day 0
Day 14, Day 30, Day 60+/-3 days
In association with 3 eligible platelet transfusions during hospitalization after transplant Immediately BEFORE platelet transfusion (concurrent CBC **NOT** required) 30-60 minutes AFTER platelet transfusion 6 hrs (+/- 60 minutes) AFTER platelet transfusion 24 hrs (+/- 60 minutes) AFTER platelet transfusion
Day 100+/ 7 days
Month 6, Month 9 +/ 14 days
Month 12, Month 24 +/ 14 days

*TPO samples collected in a large red top tube. Collect a CBC concurrently with each TPO sample unless otherwise specified. Since these assessments are entirely voluntary and optional, there will be no protocol deviations or violations for missed samples.

Appendix 2: Functional Reconstitution of T cell Immunity

15 ml of blood in yellow top tubes will be collected at the following time points

1. Pre Transplant
2. Days 30 +/- 3 days, 60 +/- 3 days, 100 +/- 7 days post transplant
3. Months 6, 9, 12, and 24 post transplant all +/- 14 days

These samples will be utilized to assess the following endpoints:

1. Thymic regeneration by assessing signal joint TCR excision circle (TREC) DNA.
2. Quantitative reconstitution of pathogen-specific effectors (specific for CMV, EBV and aspergillus) by using pathogen/MHC-specific pentamers.
3. Qualitative immune reconstitution by assessing function of pathogen-specific effectors by ELISpot.
4. Quantitative reconstitution of leukemia-specific effectors by using WT1/MHC-specific pentamers.
5. Induction of allo-specific anergy vs. GvHD.

These studies will be done in the laboratory of Dr. Vicki Boussiotis.

The tubes will be transported by courier from MGH and hand picked up at DFCI and BIDMC.

Appendix 3: Immune monitoring

A secondary endpoint in this protocol is to monitor immune reconstitution after stem cell transplantation and to compare results with similar patients treated in previous protocols in which ATG was used in the conditioning regimen. In order to analyze this endpoint the following samples will be collected at the time points described below:

30 ml of blood will be collected in 3 lavendar top tubes (EDTA anti-coagulant) at the following time points:

1. Pre Transplant
2. Days 14+/- 3 days, 30 +/-3 days, 60+/- 3 days, 100 +/-7 days post transplant
3. Months 6, 9, 12, and 24 post transplant, all +/- 14 days

These samples will be transported to the Pasquarello Tissue Bank(Dr. Jerome Ritz) at the Dana Farber Cancer Institute at the following address:

Appendix 4: Performance Status

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Description	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed < 50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Appendix 5: Suggested Guidelines for Cord Blood Selection

1. Cord blood search should be initiated as the time of MUD search. Patients who have a readily available and eligible 8/8 A, B, C, DR matched unrelated donor or a 8/8 or 7/8 (A, B, C, DR) related donor are not eligible for our cord blood studies. All available siblings and usually adult children and healthy parents should also be typed. Mismatches at DQ are acceptable.
2. Patients who have an unfavorable MUD search (less than 10 likely 10/10 allele matched donors or obvious linkage disequilibrium issues) should have cord units selected for high resolution typing.
3. The NMDP, BMDW, and all cooperative registries should be searched simultaneously.
4. HLA antibodies should be checked at the time of cord blood search—we should not select UCB units against which patients have preformed antibodies. Antibody screen may need to be repeated just prior to ordering cords.
5. Cord units must not be HLA identical, unless a 6/6 match with the patient.
6. Double mismatches at any given locus should be avoided
7. Cord blood units should have high resolution typing performed for Class I and Class II. This can be done from an attached segment or stored DNA as available. A, B, and DR are used in the match strategy.. Cord unit to patient matching should be at the allele level (high resolution) whenever possible. Data on C and DQ are collected but not used in the match strategy. Is possible, cord blood identity should be confirmed just prior to transplant on an attached segment.
8. Cord units are very expensive. Therefore, cord blood units should not be ordered until the patient has signed consent, has insurance approval, and has met eligibility criteria for transplant.
9. Patients should sign the consent for NMDP IND, and when approved, from the New York Blood Center, at the time of search, which grants permission to use UCB units not licensed yet by the FDA. This consent must be signed in addition to any research consents.
10. Patients must have an accurate weight at the time of search.
11. Choices regarding cell dose vs. HLA for double cord shall be as follows:

    - Total TNC must be at least 3.7×10^7 NC/kg
    - Each unit must be at least 1.5×10^7 NC/kg
    - Higher cell dose triumphs over HLA match when TNC is $\leq 2.0 \times 10^7$ NC/kg for each single CBU
    - Closer HLA match triumphs over cell dose when TNC is $>2.0 \times 10^7$ NC/kg for each single CBU unless there is a >50% higher cell dose in the less well matched cord blood unit

12. For selection between equivalent units choose:
    - CD 34+ dose (higher dose preferable)
    - Greater viability (never use a unit with viability less then 90%)

.

Example:
Cord A 4/6 match to patient, cell dose 3 x 10^7 NC/kg
Cord B 5/6 match to patient, cell dose 1.7 x 10^7 NC/kg
Cord C 4/6 match to patient, cell dose 5.0 x 10^7 NC/kg
Select A and C

Example:
Cord A 4/6 match to patient, cell dose 3.5 x 10^7 NC/kg
Cord B 5/6 match to patient, cell dose 3.0 x 10^7 NC/kg
Cord C 4/6 match to patient, cell dose 5.0 x 10^7 NC/kg
Select B and C

Appendix 6. Suggested Management of HHV-6 Infection.

1. If encephalitis is present, Foscarnet should be instituted immediately, even in the absence of results from blood or CSF HHV-6 levels.

2. Perform lumbar puncture; if negative for HHV-6, discontinue Foscarnet. If positive, continue Foscarnet.

3. If no encephalitis but the HHV-6 blood viral load is >100, 000 copies institute Foscarnet.

4. Asymptomatic patients with HHV-6 viral loads <100,000 copies often do not require treatment.