

Protocol Number: RP 01-05



Protocol Title:

**NON-MYELOABLATIVE ALLOGENEIC HEMATOPOIETIC STEM CELL
TRANSPLANTATION FOR HEMATOLOGIC MALIGNANCIES AND
DISORDERS.**

Roswell Park Study Number: RP 01-05

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1.0) INTRODUCTION:

- 1.1) Conventional allogeneic stem cell transplantation is a valuable approach to therapy for many hematologic malignancies. However, high dose conditioning regimens are associated with a high incidence of acute and long-term side effects. This has precluded the use of allografting for older patients and those patients with pre-existing organ damage. Thus, an important goal is the development of safer allografting procedures that can be extended to those patients who would otherwise suffer from a high morbidity and mortality during allogeneic transplant.

Considerable data support the presence of a graft versus leukemia (GvL) effect after allogeneic transplants, including the reduced risk of leukemic relapse in patients with acute and chronic graft versus host disease (1,2,3). There is a higher risk of leukemia relapse after syngeneic (identical twin) bone marrow transplantation (4,5,6) and after T-cell depleted allotransplants due to lack of a graft versus leukemia (GvL) effect (1,7). The most striking and direct evidence supporting the existence of the GvL effect is the observation that certain patients who relapse after allogeneic transplantation can be re-induced into complete remission by infusing additional donor lymphocytes (8,9,10,11,12). The data discussed above indicate that the graft versus leukemia/lymphoma (GvL) effect alone may cure susceptible diseases. An alternative strategy is to utilize a less intense, non-myeloablative conditioning regimen designed not to eradicate the malignancy but to provide sufficient immunosuppression to achieve donor engraftment. This strategy allows subsequent development of a graft versus malignancy effect.

Recent preclinical studies in a canine model have shown that the conditioning regimen for allografting can be reduced in intensity yet still achieve the goal of engraftment (13). Other investigators have performed pilot trials to determine whether this strategy of non- myeloablative chemotherapy with allogeneic blood stem cell transplantation could induce durable engraftment and remission in patients with hematologic malignancies. This approach was studied in patients who were ineligible for standard myeloablative conditioning regimen because of advanced age or co-morbid illness.

Childs et al at NIH investigated lineage specific chimerism in fifteen patients receiving an allogeneic peripheral blood stem cell transplant from an HLA-identical (n=14) or a 5/6 HLA antigen matched sibling donor after a preparative regimen of cyclophosphamide and fludarabine (14). Eight patients had hematologic malignancies, MDS (n=3), CML chronic phase (n=2), CML second chronic phase (n=1)), refractory extramedullary plasmacytoma (n=1), relapsed diffuse large cell non-Hodgkin's lymphoma (n=1). Seven patients had solid tumors, metastatic

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melanoma (n=4), metastatic renal cell ca (n=3). Seven of 14 patients had mixed (donor range; 40% to 90%) T-cell chimerism on day +30. After cyclosporine A (CSA) withdrawal, three additional patients became 100% donor T-cell chimeric. The four remaining patients with mixed T-cell chimerism, 2-4 weeks after CSA withdrawal received 1-3 doses of donor lymphocyte infusions (DLI) given at 30 day intervals. Three achieved 100% donor T-cell chimerism. One patient, who only reached a maximum of 40% donor T-cell chimerism, showed a progressive decrease in percentage of donor T-cells despite three doses of donor lymphocyte infusions (2, 10 and 150 x 10⁶ CD3⁺ cells/kg) and completely rejected the transplant by day +72. Overall, 13 out of 14 patients established 100% donor T-cell chimerism. At day +30 posttransplant, all 14 surviving patients had evidence of residual disease. Subsequently, ten patients (71.4%) had disease regression, five of whom remain in complete remission (CR). One patient with extensive renal cell cancer and two patients with CML remain disease free 13, 12, and 5 months, respectively, post transplant.

Indolent lymphoid malignancies are also affected by graft versus malignancy effects (15). Fifteen patients with chronic lymphocytic leukemia (CLL) or lymphoma have been treated using a non-myeloablative regimen of fludarabine or cyclophosphamide or fludarabine, cytarabine, and cisplatin (16). All patients had failed to respond or had disease after primary chemotherapy. Nine patients had CLL in relapse after a prior fludarabine response, and 6 patients had lymphoma. All patients had active disease at the time of transplantation. Three had an ECOG performance status of 3, two patients had elevated liver function tests, and all had received extensive prior therapy. Eleven of 15 patients had evidence of engraftment. The percentage of donor cells in marrow ranged between 50% and 100% at one month after transplantation. One patient had 75% donor cells in his marrow at 6 weeks post-transplant and converted to 100% donor cells following donor lymphocyte infusion (DLI). All eleven patients with engraftment had a response, and 8 patients achieved complete remission. Maximal responses are slow to develop and occur gradually over a period of several months to a year.

High dose therapy with allogeneic marrow transplantation in multiple myeloma carries a high risk of treatment related mortality, up to 70% in some series (17,18,19). The existence of a graft versus myeloma effect is also well established, as indicated by the success of DLI in re-inducing remission in selected patients relapsing after allogeneic transplant (20,21). The strategy of a non-myeloablative conditioning regimen is of major interest as a potential means of harnessing this graft-versus-myeloma effect while reducing regimen related toxicities.

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Fanconi anemia is an autosomal recessive disorder characterized by chromosomal fragility, congenital abnormalities, progressive bone marrow failure and a marked propensity to develop malignancies. FA is only cured by hematopoietic stem cell transplantation, and is a universally accepted indication for HLA-identical sibling allografts (38). FA patients are natural candidates for reduced intensity conditioning because of their cellular hypersensitivity to DNA crosslinking agents such as cyclophosphamide and radiotherapy (39). Fludarabine based regimens are attractive as they are capable of intense T-cell immunosuppression, and have been used successfully for nonmalignant diseases, including Fanconi anemia, with minimal toxicity and stable, durable engraftment (40, 41).

This transplant approach has advantages over conventional transplant; first, acute GVHD is delayed and usually occurs after patients have fully recovered from conditioning-related toxicities. Second, the ability to modify chimerism by withdrawing anti-GVHD medications or by DLI allows flexibility in selecting the optimum pace of donor immune recovery, with its attendant benefit from graft versus tumor effects and its risk of GVHD induction. While these low intensity transplants have a decreased immediate procedural mortality, the long-term efficacy must still be determined. Furthermore, because the preparative regimen does not contribute a significant anti-malignancy effect, the risk of disease progression might be higher.

2.0) OBJECTIVES:

2.1) Primary objectives are:

2.1.1) To determine safety and toxicity of this form of treatment in a patient population that is usually not eligible for full myeloablative allogeneic transplant

2.2) Secondary objectives:

2.2.1) To evaluate clinical response and overall outcome of non-myeloablative allogeneic hematopoietic stem cell transplantation/ cord blood transplantation.

2.2.2) To evaluate the evidence of graft-versus-tumor effect/ graft-versus-host disease/ chimerism of non-myeloablative allogeneic stem cell transplantation for the treatment of transplantable hematological malignancies and disorders.

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3.0) ELIGIBILITY CRITERIA:

- 3.1)** Diagnosis of disorders or histologically documented hematologic malignancy or transplantable disorder.
- 3.2)** Patients not eligible for standard myeloablative allogeneic stem cell transplant.
- 3.3)** Disease Status:

3.3.1) Hematologic Disorders:

Severe disorders as described in literature (22). Failed at least one cycle of standard immunosuppressive regimen with cyclosporine (CSA) and anti-thymocyte globulin (ATG).

Life-threatening disorders for which there is no standard therapy e.g. congenital bone marrow disorders (including congenital dyserythropoiesis, thrombasthenia that could lead to life threatening conditions.

3.3.2) Acute Leukemia:

3.3.2.A) Resistant or recurrent disease after combination chemotherapy with at least one standard regime (23, 24, 25).

3.3.2.B) First remission patients at high risk of relapse.

- AML - antecedent myelodysplastic syndrome, secondary
- AML, high risk cytogenetic abnormalities (23).
- ALL - high-risk cytogenetic abnormalities (25).

3.3.3) Chronic Myeloid Leukemia (CML):

- Chronic phase (failed Gleevec, failed interferon after at least 6 months of treatment with minimum of 21 million units of Interferon/ week or unable to tolerate Interferon).
- Accelerated Phase (blasts < 20%).

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3.3.4) Myeloproliferative and myelodysplastic Syndromes:

- Myelofibrosis (after splenectomy).
- Refractory anemia.
- Refractory anemia with excess blasts.
- Chronic myelomonocytic leukemia.

3.3.5) Lymphoproliferative disease:

- CLL, Low-grade non-Hodgkin Lymphoma (recurrent or persistent). Symptomatic disease after first-line chemotherapy.
- Multiple myeloma (progressive disease after autologous stem cell transplant).
- Waldenstrom's macroglobulinemia (failed one standard regimen).

3.3.6) Non-Hodgkin Lymphoma: intermediate and high grade:

- Controlled and chemo-sensitive disease.
- First remission lymphoblastic or small, non-cleaved cell lymphoma at high risk of relapse (26,27).

3.3.7) Hodgkin's disease:

- Relapsed and chemosensitive disease.

3.3.8) Fanconi anemia:

- Positive chromosome breakage analysis using diepoxybutane (DEB)

3.4) Identification of suitable donor:

- Donor eligibility criteria is outlined in section 5.0

3.5) Age ≥ 4 , ≤ 75 years for related and fully matched blood and bone marrow unrelated donor transplants and ≤ 60 for unrelated cord blood transplants.

3.6) No serious concomitant psychiatric illness.

3.7) No concomitant malignancy other than non-melanoma skin cancer.

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- 3.8)** Non-pregnant and non-nursing woman. (women and men of reproductive potential should agree to use an effective means of birth control).
- 3.9)** Patients may have received prior autologous BMT.
- 3.10)** Patients may have received prior allogeneic BMT (at least 6 months have elapsed).
- 3.11)** Informed consent.
- 3.12)** ≥ 2 weeks since prior chemotherapy, radiation treatment and/or surgery.

4.0) EXCLUSION CRITERIA:

- 4.1)** Uncontrolled CNS disease (for hematologic malignancies).
- 4.2)** Karnofsky performance status $\leq 50\%$.
- 4.3)** DLCO less than 40% predicted, corrected for Hb and/or alveolar ventilation.
- 4.4)** Cardiac ventricular ejection fraction (MUGA scan) less than 35%.
- 4.5)** Bilirubin, alkaline phosphatase, SGOT/SGPT ≥ 3 x institutional normal.
- 4.6)** Child's class B and C liver failure (see appendix no. VII).
- 4.7)** Calculated creatinine clearance ≤ 40 cc/min.
- 4.8)** Uncontrolled diabetes mellitus, cardiovascular disease, active serious infection or other disease which in the opinion of treating physician, would make this protocol unreasonably hazardous for the patient.
- 4.9)** HIV antibody positive.

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5.0) DONOR ELIGIBILITY CRITERIA/ STEM CELL SOURCE CRITERIA:

- 5.1)** HLA matching is important in determining the risk for transplant related complications, in particular GVHD. The HLA loci: A, B, DR and to lesser extent C have been identified as regions that are important in determining HLA matching. Full HLA identity is associated with the least risk of GVHD. Initially matching was determined at the A, B, DR loci with a total of 6 possible alleles (3 inherited from each parent). A single antigen mismatch at A, B, or the DR transplant from a family member is associated with a higher risk of GVHD but similar survival when compared to full identity at these 3 regions. When evaluating patients for unrelated donor transplant, a higher degree of matching is preferred due to the higher risk of GVHD. However, recent data from the National Marrow Donor Program has demonstrated that single antigen mismatching with a reduced-intensity transplant results in acceptable levels of GVHD.

Molecular testing has allowed for more detailed analysis of the HLA locus (34). Multiple alleles have been described in the A, B, DR and C loci. The DR region has been further defined by molecular typing into 2 major regions: DRB1 and DQB1. However the clinically relevant antigen for matching is DRB1. In addition, molecular testing has allowed for more accurate determination of the C region. Thus, matching can take place at a more stringent level for unrelated donor transplants.

Related donor/recipient pairs must be matched at 6 or 5 HLA antigens (A, B, DR). Volunteer unrelated donor/recipient pairs must be matched at the antigen level by molecular analysis at the DRB1 loci for blood and marrow transplants. An antigen mismatch may be considered in patients otherwise matched at the other loci. Patients who do not have an appropriate unrelated donor will be considered for cord blood transplant on this protocol.

- 5.2)** Single antigen mismatch at A, B, or C may participate in this protocol for voluntary unrelated donors. The priority would be C mismatching followed by A then B.
- 5.3)** If a patient has no suitable family donor matched for 5 or 6 HLA antigens (A, B, DR) and no suitable unrelated donor is identified, patient can be considered a candidate for cord blood transplant, provided a cord blood donor is identified either a 4,5 or 6 match with the patient for HLA antigens (A, B, DR). The cord blood product must provide a minimum of 2×10^7 nucleated cells/kg, test negative for HIV and Hepatitis A, B and C, and sterility assays have no growth

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(28,29,30,31,32). The cord blood products are located through the National Marrow Donor Program, the American Registry, or the Bone Marrow Donor Worldwide, and may be stored in the N.Y Placental Cord Blood Bank, the St. Louis Cord Blood Bank, or any of the established, registered European or Canadian blood and marrow banks.

- 5.4) The donor must be healthy and must have negative testing for HIV, hepatitis and syphilis. Donors who test positive for hepatitis and/or syphilis must be cleared by infectious disease consultation.
- 5.5) The donor must have no uncontrolled cardiopulmonary, renal, endocrine, hepatic or psychiatric disease.
- 5.6) Donor must be able to give informed consent for peripheral blood stem cell collection or bone marrow collection.
- 5.7) Syngeneic donors are not eligible.
- 5.8) Donors who have no good peripheral venous access, may require central venous line placement for stem cell collection.

6.0) REGISTRATION AND DATA SUBMISSION:

6.1) Contact the BMT coordinator.

6.2) Registration requirements:

6.2.1) Informed consent: Patient must be aware of the neoplastic nature of **his/her disease and willingly consent after being informed of the** procedure to be followed, the experimental nature of the therapy, alternatives, potential benefits, side-effects, risks, and discomforts. Human protection committee approval of this protocol and of its consent form is required.

6.2.2) Histological review: Submission of appropriate tissue sample to confirm the underlying diagnosis.

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6.2.3) Radiological review: All patients with lymphoma/ multiple myeloma must have appropriate radiographic workup at least 4 weeks prior to registration and must be submitted for review.

6.2.4) Registration procedures: Confirm eligibility criteria (see Sections 4.0 & 5.0). Complete the registration worksheet. When the patient is registered, a patient identification number will be generated. Data pertaining to this protocol will be collected by the BMT research nurse, and/ or any other persons assigned by the BMT department head.

7.0) REQUIRED DATA:

7.1) Protocol Date Definitions:

On-Study Date	Start Treatment Date	Stop Treatment Date	Off-Study Date
Date conditioning regimen started	Date conditioning regimen started	Date infusion of stem cells is complete (relevant for patients who have more than one infusion over 2 or more days)	Date of first disease progression post-transplant or date of death due to any cause, date patient failed to engraft, or 4 years of follow up

7.2) To be completed within 4 weeks before registration. (see appendix VIII)

7.3) Record on the flow sheets:

Patient name
Patient identification number
Date of birth
Patient's gender
Disease type and stage
Treatment start date
Date of signed consent
Patient's race

- Important hematologic recovery endpoints;
Day ANC $\geq 500/\mu\text{L}$; Plt $\geq 20\text{K}$.
Day of last Platelet transfusion, last RBC transfusion.
- Day of occurrence of severe toxicity (grade 3) by Bearman criteria.

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8.0) TREATMENT PLAN:

8.1) Stem cell Collection:

Donors will receive 10 micrograms/ kg GCSF daily sub-cutaneously, starting on day -5. Mobilized peripheral blood stem cells (PBSCs) will be collected by apheresis on day “-1” and again on day “0” if necessary, to achieve a minimum target dose of 2×10^6 CD34⁺ cells/kg of recipient weight (maximum 8×10^6 CD34⁺ cells /kg for related donors, no maximum for unrelated donors) (33, 34). PBSCs will be unmanipulated. CD3⁺ and CD34⁺ cells as fractions of the peripheral mononuclear cells (PMN) will be determined from the established PMN cell collection according to institutional flow cytometry.

The preferred method of collection is peripheral blood collection, but if peripheral blood collection either cannot be obtained or this method is declined, a bone marrow harvest will be performed. The target dose for bone marrow harvest will be 3×10^8 nucleated cells per kilogram of patient body weight. Bone marrow will be unmanipulated.

The peripheral mononuclear cells will be separated and collected, while all the other separated blood components will be returned to the donor.

If donor lives outside of the area or is unable to come back after transplantation, donor lymphocytes will be collected by apheresis before the GCSF mobilization, aliquotted into CD3⁺ cell doses as per section 8.6 and cryopreserved for subsequent thawing and infusion. If donor will be accessible after transplant, donor lymphocytes will be collected by apheresis after transplant. If the stem cell collection is $\leq 1 \times 10^6$ per kg of body weight then bone marrow harvest will be done.

8.2) Preparative regimen:

8.2.1) Preparative regimen for related and unrelated non-cord blood transplant;

Day	-5	-4	-3	-2	-1	0
Cyclophosphamide 50mg/kg IV	X	X				
Fludarabine 25 mg/m ² IV	X	X	X	X	X	
Stem cell infusion						X

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- 8.2.1.A)** Cyclophosphamide; 50 mg/kg IV on days -5 and -4. Begin IV hydration at 150 ml/m²/hr. Cyclophosphamide, 50mg/kg (ideal body weight, but if actual body weight is 25% > ideal body weight then adjusted ideal body weight will be used. Appendix II). If the actual body weight is less than ideal body weight, the actual body weight will be used. The cyclophosphamide will be mixed in 500ml of an appropriate IV solution, is given by 2-hr IV infusion (e.g., 1100-1300 hrs) on day -5 and -4 (total dose 100 mg/kg). Hyperhydration is continued until 24 hrs, after the last cyclophosphamide dose. Lasix, 10-20 mg IV, will be given 2 hrs after each cyclophosphamide dose and then every 4 hrs. as needed to maintain urine output \geq 100ml/hr.
- 8.2.1.B)** Fludarabine, 25 mg/m² (actual body weight) IV on days -5, -4, -3, -2, and -1. Fludarabine, 25 mg/m² in 100 ml of appropriate IV solution, is given by 30-minute IV infusion (e.g., 0900 – 0930 hrs.) on days -5, -4, -3, -2 and -1 (total dose 125 mg/m²).

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8.2.2) Preparative regimen for cord blood transplant:

Day	-5	-4	-3	-2	-1	0
Cyclophosphamide 50mg/kg/d IV	X	X				
Fludarabine 25 mg/m ² /d IV	X	X	X	X	X	
*Anti-thymocyte Globulin. (ATG) 30mg/Kg/d IV			X	X	X	
Stem cell infusion						X

*ATG will be given as preparative regimen for cord blood transplant in addition to Cyclophosphamide and Fludarabine (as described above). Start ATG 2 hours after CTX completion. ATG is given at 30mg/Kg IV on day -3, -2 and -1. Acetaminophen (10mg/Kg or 650 mg, max), Diphenhydramine (1.25 mg/Kg IV or 50 mg max), and Methylprednisolone (2 mg/Kg) IV will be given prior to each ATG dose. ATG is infused over 4 hrs. **(Use ideal body weight for ATG, but if actual body weight is > 25% ideal body weight, then adjusted ideal body weight will be used. Refer to Appendix II. If the actual body weight is less than ideal body weight, the actual body weight will be used.)**

8.2.3) Preparative regimen for patients with Fanconi anemia (matched related, matched unrelated, or cord blood transplant)

Day	-6	-5	-4	-3	-2	-1	0
Cyclophosphamide 7.5 mg/kg/d IV	X	X	X	X			
Fludarabine 25 mg/m ² /d IV	X	X	X	X	X		
Anti-thymocyte globulin (ATG) 30 mg/kg/d				X	X	X	
Stem cell reinfusion							X

8.2.3A) Cyclophosphamide: 7.5 mg/kg/d on days -6, -5, -4 and -3. Begin IV hydration at 3000 ml/m²/day. Start hydration 12 hours before cyclophosphamide and continue for minimum of 24 hours after last dose of cyclophosphamide. Use actual body weight, or adjusted ideal body weight if actual > 25% of ideal weight (if actual less than ideal use actual weight, see Appendix II). Cyclophosphamide is given as a 2 hour infusion each day (e.g., 0930-1130). Dilute in an appropriate IV solution and

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run over 2 hours). Lasix 0.5 mg/kg (no max. dose) IV will be given every 2 hours as needed to maintain urine output at minimum of 2 cc/kg/hr. Recommended max dose is 20-40 mg.

8.2.3B) Fludarabine on days -6, -5, -4, -3, and -2. Base on actual body weight. Dilute in 100ml (or 50 ml if < 1m²) of appropriate IV solution and run over 30 minutes (eg, 0900-0930).

8.2.3C) ATG is given at 30 mg/kg IV on days -3, -2, and -1. Acetaminophen (10-15 mg/kg po, max 650 mg), Diphenhydramine (1-1.25 mg/kg IV, max 50 mg), and methylprednisolone (2 mg/kg IV) (**ideal body weight, but if actual body weight is > 25% of ideal body weight then adjusted ideal body weight will be used. Refer to Appendix II. If the actual body weight is less than ideal body weight, the actual body weight will be used**) will be given as premedication 30 minutes before each dose of ATG. Continue acetaminophen every 4 hours and diphenhydramine every 6 hours until 24 hours after completing ATG. Infuse ATG over 4 hours.

8.3) GVHD Prophylaxis:

8.3.1) GVHD prophylaxis for related and unrelated blood and marrow transplant (35):

DAY	-1	0	+1	+2	+3	+4	+5	+6
FK506 ^ψ	X	X	X	X	X	X	X	X
MMF ^Σ	X	X	X	X	X	X	X	X
MTX ^Ω			X		X			X

^ψ Tacrolimus (FK506): Dose will be 0.03 mg/kg (ideal body weight) PO BID or IV equivalent, from day -1 to day +60 then taper as described in section 8.6.1. The target level of tacrolimus will be 5-15 ng/ml. In patients who are unable to take oral tacrolimus, the intravenous dose is generally 1/3 of the oral dose. Levels of FK506 will be maintained in the therapeutic range. Use ideal body weight or Actual weight if less than ideal body weight.

^Σ Mycophenolate mofetil (MMF): Dose will be 1 gram by mouth (PO) or IV twice a day (37,38). MMF will be continued from day -1 to day + 60, and then taper as described in section 8.6.1. The pediatric dose will be 600mg/m² BID. Patients with a BSA of 1.25 to 1.5 can be started on a dose of 750 mg BID; those with a BSA > 1.5 can be dosed with 1 GmBID.

^Ω Methotrexate 5 mg/m² IVPB on days 1, 3 and 6

8.3.2) GVHD prophylaxis for cord blood transplant:

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DAY	-1	0	+1	+2	+3	+4	+5	+6
FK506 ^Ψ	X	x	x	x	x	x	x	x
MMF ^Σ	X	x	x	x	x	x	x	x
Methylprednisolone (0.5mg/kg BID) ^Θ			X	X	X	X		
Methylprednisolone (1 mg/kg BID)							X	X

- Ψ Tacrolimus (FK506); Dose will be 0.03 mg/kg (ideal body weight) PO BID or IV equivalent, from day -1 to day +180 then taper as described in section 8.6.1. The target level of tacrolimus will be 5-15 ng/ml. In patients who are unable to take oral tacrolimus, the intravenous dose is generally 1/3 of the oral dose. Levels of FK506 will be maintained in the therapeutic range.
- Σ Mycophenolate mofetil (MMF); Dose will be 1 gram by mouth (PO) or IV twice a day. MMF will be continued from day -1 to day + 60, and then taper as described in section 8.6.1. Refer to 8.3.1 for dosing in pediatric patients.
- Θ Methylprednisolone (ideal body weight, but if actual body weight is > 25% ideal body weight then adjusted ideal body weight will be used. Refer to Appendix II. If the actual body weight is less than ideal body weight, the actual body weight will be used.) will be given at a dose of 1 mg/kg IV (0.5 mg/kg BID) from day +1 to day +4 and 2 mg/kg (1 mg /kg IV BID) beginning on day +5 until day +19 or until the first day ANC reach $\geq 500/\text{mm}^3$. After ANC have reached $\geq 500/\text{mm}^3$, steroids should be tapered by 0.2 mg /kg/week.

8.3.3) GVHD Prophylaxis for Patient with Fanconi's anemia

8.3.3A) Tacrolimus (FK506): Dose will be 0.03 mg/kg (ideal body weight) PO BID or IV equivalent, from day -1 to +100 for related, or 180 days for unrelated, cord blood recipients. The target level will be 5-15 ng/ml. In patients unable to take oral medication, the IV dose is 1/3 the oral dose. Taper if no signs of GVHD by 10% per week.

8.3.3B) Mycophenolate mofetil (MMF): Dose will be 600 mg/m² po or IV BID for patients < 1.25 m². For patients with BSA 1.25-1.5, dose will be 750 mg po or IV BID. For patients with BSA > 1.5 m², dose will be 1000 mg (1 gm)

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po or IV. Dosing will start on day -1 and continue until day + 60. Dose will then be stopped.

8.4) Supportive care:

8.4.1) Prior to initiating therapy, placement of a multi-lumen indwelling silastic catheter is required.

8.4.2) Patients will receive full supportive care, including transfusions of blood and blood products, erythropoietin, antibiotics, anti-emetics, etc., when appropriate. The reason(s) for treatment, dosage, and the dates of treatment should be recorded on the flow sheets.

8.4.3) Use of allopurinol will be at the discretion of the treating physician.

8.4.4) Mucosal evaluation and care: Mucositis is expected to be very mild with this chemotherapy regimen. Stomatitis and esophagitis due to herpes virus may be confused with drug-induced mucositis and viral cultures should be obtained frequently. Patients should receive acyclovir according to the RPCI standard of care for an allogeneic transplant patient.

8.4.5) Candida prophylaxis: Antifungal prophylaxis should be given according to the RPCI standard of care for an allogeneic transplant patient.

8.4.6) Pneumocystis Pneumonia (PCP) Prophylaxis: Anti-PCP prophylaxis should be given according to the RPCI standard of care for an allogeneic transplant patient

8.4.7) CMV Infections: No prophylaxis for CMV will be initiated. Surveillance for CMV using CMV antigenemia is required once weekly until day +100 and then PRN (as needed). Patients with positive CMV antigenemia should receive treatment as per the RPCI SOP. Dosing will be adjusted for hematological and renal toxicity. Maintenance therapy will be left up to attending physician's discretion. CMV surveillance will start no later than day +21 unless patient has not engrafted.

8.5) Allogeneic stem cell reinfusion:

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8.5.1) On Day 0 a minimum total cell dose of (CD34⁺ cells) $2 \times 10^6/\text{kg}$ (blood) No maximum CD34⁺ dose for blood.) An adequate minimum CB dose is 2×10^6 nucleated cells/kg. BM dose is 1×10^8 nucleated cells/kg.

8.5.2) GM-CSF 250 $\mu\text{g}/\text{m}^2$ subcutaneously daily beginning on day +7 and continuing until $\text{ANC} \geq 500/\mu\text{L}$ for three consecutive days. If the ANC falls to $\leq 500/\mu\text{L}$ then resume GM-CSF at 250 $\mu\text{g}/\text{m}^2$. G-CSF may be substituted if patients are unable to tolerate GM-CSF.

8.6) Followup;

8.6.1) Immunosuppression:

After transplant, serial samples of blood and marrow will be analyzed for chimerism analysis by polymerase chain reaction (PCR). Chimerism will be analyzed in blood on day 30 - 40, day 60 - 70 and then as indicated in section 8.6.3. Chimerism will be analyzed in bone marrow (for leukemia and patients with other malignancies if bone marrow was involved previously) on day 30- 40 and day 100 -120 and then as indicated in section 8.6.3.

For peripheral blood and bone marrow transplant patients, those showing 100% donor T-lymphocyte chimerism by minisatellite analysis on day +30 continue FK506 and MMF until day +60; thereafter MMF will be stopped. Cord blood patients will be continued on FK506 until day + 180. All patients will be tapered off FK506 by 25% every 10 days and discontinued if no GVHD develops. Patients not converting to 100% donor T-cell chimerism by day + 120 and showing signs of progression of disease after FK506 and MMF withdrawal, will be evaluated for DLI (section 8.6.5). Pts who receive cord blood will not receive DLI. For Fanconi Anemia, taper FK506 at day 100 (for related donor) or day 180 (for unrelated donor).

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8.6.2) Disease measurement:

Patients will be followed according to response criteria as referenced in Section 11.4.

8.6. 3) Management of acute graft versus host disease;

Patients with symptomatic grade 1 acute GVHD of the skin will be treated with topical steroids. Grade II or greater acute GVHD will be treated with high dose methylprednisolone 1-2 mg/kg daily for 10-14 days followed by slow tapering in responders. At 10% weight, continue for at least 2 weeks from the disappearance of all symptoms of GVHD. FK506/MMF will be maintained or increased during active aGVHD. Refractory aGVHD will be treated as per attending physician's discretion.

8.6.4) Donor Lymphocyte Infusion;

8.6.4.A) Eligibility Criteria for Infusion of Donor Lymphocytes:

- Patients have either persistent AML/ALL or progressive disease (Lymphoma/multiple myeloma).
- Patients must be off all immunosuppressives for a minimum of 30 days.
- Patients must show no signs of active GVHD
- The original donor must be available

8.6.4.B) Collection of Donor Lymphocytes:

The original donor will undergo leukapheresis to collect 1.0×10^7 CD3⁺ cells/kg (recipient weight) for use as donor lymphocyte infusions.

8.6.4.C)

Infusion of Donor Lymphocytes: 1×10^7 CD3⁺ cells/kg from the original donor.

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8.6.4.D)

Prophylaxis for GVHD and post-transplant immunosuppression will not be given at this time.

8.6.4.E)

Patients will be monitored weekly for engraftment and toxicity. Sample submission for chimerism studies should be collected on day +30 after DLI. Samples should be collected prior to an infusion of donor lymphocytes.

8.6.5) Second Infusion of Donor Lymphocytes (4 weeks after first DLI).

8.6.5.A) Eligibility Criteria for a Second Infusion of Donor Lymphocytes:

- Patients have either persistent AML/ALL or progressive disease (Lymphoma/multiple myeloma).
- Patients must be off all immunosuppressives for a minimum of 30 days.
- Patients must show no signs of active GVHD
- The original donor must be available

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8.6.5.B)

Collection of Donor Lymphocytes; The original donor will undergo leukapheresis to collect 5×10^7 CD3⁺ cells/kg (recipient weight). Alternatively, cells from the initial stem cell collection may be stored and frozen.

8.6.5.C)

Infusion of Donor Lymphocytes: 5×10^7 CD3⁺ cells/kg from the original donor will be infused over 2 hours.

8.6.5.D)

Prophylaxis for GVHD and post-transplant immunosuppression will not be given at this time.

8.6.5.E)

Patients will be monitored weekly for engraftment and toxicity with chimerism samples collected again on day +30 after DLI. Samples should be collected prior to an infusion of donor lymphocytes.

8.6.6)

Third Infusion of Donor Lymphocytes: If more than 8 weeks from second DLI and all criteria for second DLI in Section 8.6.4.A are met, an optional infusion of 5×10^7 CD3⁺ cells/kg from the original donor may be administered.

9.0) POTENTIAL TOXICITIES, THEIR MANAGEMENT AND DOSE MODIFICATION:

9.1) REGIMEN RELATED TOXICITY (Bearman Criteria, Appendix 1).

9.1.1) Cardiac:

The risk of fatal cardiac toxicity with the study regimens is <5%. Patients who develop signs of congestive heart failure not attributable to fluid overload should be evaluated for signs of carditis before additional

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cytoxan is given. Studies should include ECG (to compare voltage to pretreatment ECG), radionuclide ventriculogram, and/or echocardiography. If evidence of pericarditis, pericardial effusion or impaired myocardial function (decreased ejection fraction) is found, no further chemotherapy will be administered. In this situation patients must be watched carefully for signs of pericardial tamponade; pericardiectomy may be required.

9.1.2 Bladder:

Urinary metabolites of cyclophosphamide can produce hemorrhagic cystitis. Microscopic hematuria is common following high dose cyclophosphamide regimens and up to 20% of patients may have gross hematuria. Vigorous hydration of patients (3,000 ml/m²/day) prior to beginning cyclophosphamide and continuing until 24 hours after the final cyclophosphamide dose, produces a dilute urine and reduces the likelihood of severe cystitis. The risk of life-threatening cystitis is estimated to be <1%. For patients with a history of previous cyclophosphamide induced hemorrhagic cystitis, MESNA may be used.

9.1.3 Renal:

All these agents in high doses can produce renal toxicity which can be minimized by vigorous hydration and avoidance of concomitant exposure to other nephrotoxic agents. Kidney damage is usually reversible, but severe cases may require dialysis.

9.1.4 Pulmonary:

With the study regimens the risk of fatal idiopathic interstitial pneumonitis is felt to be <10%. Patients with diffuse pulmonary infiltrates often present a difficult diagnostic and management problem. Whenever possible, histologic confirmation of the diagnosis should be attempted by bronchoalveolar lavage, transbronchial or open-lung biopsy to exclude infectious causes, including cytomegalovirus.

9.1.5 Hepatic:

Moderate toxicity is common. More severe, possibly fatal, liver toxicity can occur, usually in the form of veno-occlusive disease. With the study regimens the incidence of fatal liver toxicity should be < 10%. Management of liver toxicity is with standard supportive and symptomatic measures. Avoid exposure of patients to other hepatotoxic agents whenever possible.

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9.1.6 CNS:

Fludarabine can cause weakness, paresthesias or peripheral neuropathies, visual or auditory impairment, and/or mental status changes such as confusion, agitation, depression, or coma. Some antibiotics, which may be needed to treat infections during the period of low white blood cell counts, can cause VIIIth cranial nerve damage resulting in hearing loss (especially high-frequency tones) and dizziness which may be permanent. To minimize this possibility, antibiotic levels will be monitored.

9.1.7) Stomatitis/Dysphagia:

Many patients will experience moderate to severe stomatitis and dysphagia. Adequate pain relief often requires parenteral narcotics.

9.1.8) Gastrointestinal:

9.1.8.A) Nausea/Vomiting: Many patients will experience moderate to severe nausea and vomiting. All patients should receive vigorous antiemetic treatment.

9.1.8.B) Diarrhea: Most patients will experience moderate-severe diarrhea, which responds to standard symptomatic therapy. Appropriate studies are needed to exclude infectious causes of diarrhea, especially *C. difficile*.

9.2) OTHER TOXICITIES:

9.2.1) Hematologic:

All blood products will be irradiated (2500 cGy) to prevent graft vs host disease. Infection prophylaxis and treatment will be given according to standard accepted medical practice. With prophylactic platelet transfusions, the risk of death from hemorrhage is <5%.

9.2.2) Skin:

Generalized erythroderma can occur with painful palms and soles and superficial desquamation at sites of mechanical trauma. Topical steroid creams may provide symptomatic relief. Severe cases may require a brief course (3-5 days) of systemic corticosteroids. Long lasting hyperpigmentation may follow resolution of the erythroderma.

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9.2.3) Hair:

Most patients will experience total (reversible) alopecia.

9.2.4) Allergy:

ATG may be associated with a serum sickness like reaction with rash, hives, pruritis, joint aches. More severe anaphylactic like reactions with bronchospasm and shock are uncommon but reported. Prior to administration of ATG, patients will be pretreated with Tylenol, Benadryl and Solumedrol. Epinephrine and resuscitation equipment will be available at the bedside during administration.

9.2.5) Graft-vs-Host Disease:

Approximately 80% of patients will exhibit some manifestation of GvHD. Severe acute GvHD (grade II-III) may occur in up to 40% of patients with an overall mortality of 20% due to GvHD and its complications. The risk of severe or fatal GvHD is increased for older patients and for patients receiving stem cells from partially-matched donors. A chronic form of GvHD is expected to occur in up to 40% of patients who survive beyond 3 months; 10% of patients may experience significant disability due to chronic GvHD that persist for months to years post-transplant. Standard measures for prevention and treatment of GvHD will be followed.

9.2.6) Secondary malignancy:

Exposure to alkylating agents increases the risk of developing leukemia or a second cancer. The incidence of leukemia is probably approximately 5%. (Vincent T. Devita; Cancer. Principles and Practice of Oncology. 6th edition, 2000).

9.2.7) Toxicities of G-CSF:

Include bone pain, headache and muscle aches. These can be treated with non-narcotic and narcotic analgesics.

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9.2.8) With apheresis the following discomforts may occur:

Tingling around mouth or in fingers, feeling cold or feeling a pressure sensation in chest due to the blood thinner (citrate) used only in the machine method. Feeling light-headed or fainting any time during or at the end of the procedure. Pain, bruising or infection at the point of the needle entry into the skin. Reaction (such as a rash) if a drug is given to which the patient is allergic. Blood loss (seldom more than one pint) because of equipment problems which make it impossible to return the blood. Air entering the blood stream causing air to go to body organs (rare risk because of safety measures used).

9.2.9) Failure to engraft:

Autologous count recovery is the most likely outcome.

10.0) DRUG FORMULATION, AVAILABILITY AND PREPARATION:

10.1) Cyclophosphamide (Cytosan[®]; CTX; CPA; Endoxan[®]; Neosar[®]; Cytosan Lyophilized[®])

10.1.1) AVAILABILITY:

Commercially available in 100 mg/10 ml, 200 mg/20 ml, 500 mg/30 ml, or in a powder for injection in 100 mg, 200 mg, 500 mg, 1 gram, and 2 gram vials.

10.1.2) PREPARATION;

Reconstitute 100 mg, 200 mg, 500 mg, 1 gram and 2 gram vials with 5, 10, 25, 50, or 100 ml of SWI or NS to give a final concentration of 20 mg/ml. Lyophilized CYTOXAN should be prepared for parenteral use by adding Sterile Water for Injection, USP, to the vial and shaking to dissolve. Further dilute in –250-500 ml of D₅W or NS for IV infusion.

10.1.3) ADMINISTRATION;

Dissolved in diluent as per pharmacy and will be administered over 2 hours. Appropriate antiemetic therapy will be used. Patients must be adequately hydrated before, during, and after administration of cyclophosphamide.

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10.1.4) STORAGE AND STABILITY;

Solutions reconstituted with SWI or bacteriostatic water are stable for 24 hours at room temperature and 6 days if refrigerated.

10.1.5) TOXICITY;

Myelosuppression: leukopenia (nadir 8-14 days), thrombocytopenia. Acute sterile hemorrhagic cystitis (patients must be well hydrated before, during, and after treatment and have adequate renal function). Syndrome of inappropriate antidiuretic hormone (SIADH). Bladder carcinomas and cellular dysplasias. Alopecia (50%). GI: anorexia, nausea, vomiting, diarrhea. Sterile phlebitis. Rare pulmonary toxicity. Gonadal abnormalities and teratogenicity. With too rapid IV push, oropharyngeal tingling, "metallic" taste, headache, urticaria, and facial flushing can occur. With high doses, cardiac toxicity.

10.2) Fludarabine Monophosphate (Fludara®);

10.2.1) AVAILABILITY:

Fludarabine monophosphate is commercially available as FLUDARA IV as a white, lyophilized powder. Each vial contains 50 mg of fludarabine phosphate, 50 mg of mannitol and sodium hydroxide to adjust pH. Store at 15-30°C (59-86°F).

10.2.2) STORAGE & STABILITY:

Reconstituted FLUDARA IV is chemically and physically stable for 24 hours at room temperature or 48 hours if refrigerated. In addition, reconstituted FLUDARA IV contains no antimicrobial preservative and thus care must be taken to assure the sterility of the prepared solutions and should be discarded eight hours after initial entry.

10.2.3) PREPARATION:

FLUDARA IV should be prepared for parenteral use only by aseptically adding Sterile Water for Injection, USP. When reconstituted with 2 ml of Sterile Water for Injection, USP, each ml of the resulting solution will contain 25 mg of Fludarabine Phosphate, 25 mg of mannitol, and sodium hydroxide to adjust the pH to 7-8.5. The product may be further diluted for intravenous administration to a concentration < 1 mg/ml in 5% Dextrose for Injection USP or in 0.9% Sodium Chloride, USP.

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10.2.4) ADMINISTRATION:

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration. Fludarabine will be delivered as a piggy-bag via an ongoing IV line, over a period of 30 minutes.

10.2.5) TOXICITY:

Myelosuppression (dose limiting toxicity), fever, nausea, vomiting, stomatitis, diarrhea, gastrointestinal bleeding, anorexia, edema, skin rashes, myalgia, headache, agitation, hearing loss, transient episodes of somnolence and fatigue, autoimmune hemolytic anemia, autoimmune thrombocytopenia, paresthesias, peripheral neuropathy, renal and pulmonary toxicity (interstitial pneumonitis). Severe fatal CNS toxicity presenting with loss of vision and progressive deterioration of mental status were encountered almost exclusively after very high doses of fludarabine monophosphate. Such toxicity has only been rarely demonstrated at the 25-30 mg dosage of fludarabine monophosphate. Very rarely described complications include transfusion-associated graft versus host disease, thrombotic thrombocytopenic purpura, and liver failure. Tumor lysis syndrome, complicating fludarabine monophosphate therapy has been observed, especially in patients with advanced bulky disease. Opportunistic infections (protozoan, viral, fungal, and bacterial) have been observed in both pre-treated patients receiving fludarabine and in individuals receiving fludarabine combined with other agents (corticosteroids, mitoxantrone, and cyclophosphamide).

10.3) LEUKINE (GMCSF):

10.3.1) Class: hematopoietic.

10.3.2) Dosage, Adult (usual); 250mcg/m²/day SC.

10.3.3) Dosage, Pediatric, (usual);

- BMT 250mcg/m²/day IV or SC (use lower doses for first dose)
- recovery 3-15mcg/kg/day IV or SC.

10.3.4) Indications:

- AML

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- peripheral stem cell mobilization
- bone marrow transplant

10.3.5) Contraindications:

- excess leukemic myeloid blasts
- hypersensitivity to GM-CSF products
- concomitant chemo- or radiotherapy

10.3.6) Adverse effects;

- arthralgia
- chills
- rash
- fever

10.3.7) Drug Interactions;

- lithium
- vincristine

10.3.8) Pregnancy category; C

10.4) Filgrastim (r-met HuG-CSF, G-CSF: Granulocyte Colony-Stimulating Factor, Neupogen®):

10.4.1) **AVAILABILITY:**

r-met HuG-CSF is commercially available in 1.0 and 1.6 ml vials containing 300 µg and 480 µg G-CSF, respectively, by Amgen.

10.4.2) **STORAGE & STABILITY;**

G-CSF is available as a sterile buffered protein solution and must be stored at 2-8°C. DO NOT ALLOW THE DRUG TO FREEZE.

10.4.3) **ADMINISTRATION;**

Each vial should be entered only once, and the remainder of the vial discarded and not re-entered a second time. The daily dose should be injected subcutaneously in one or two sites. Standard dosing is 5 µg/kg daily as a subcutaneous injection. Higher dosing (10 µg/kg) will be used for donors in this protocol.

10.4.4) **TOXICITY:**

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Chills, nausea, anorexia, myalgias, bone pain, local injection site pain or inflammation, abnormal liver function tests, thinning of hair, and enlargement of the spleen. Rarely fluid retention and pericardial effusion are observed. All of these are generally reversible when the drug is discontinued.

10.5) Tacrolimus (Prograf®);

10.5.1) AVAILABILITY:

Tacrolimus is a commercially available macrolide compound with potent immunosuppressant properties. Tacrolimus is available for oral administration as capsules, containing the equivalent of 0.5 mg, 1 mg, or 5 mg of anhydrous tacrolimus. For IV use, tacrolimus is available as a sterile solution in 1mL ampules containing the equivalent of 5 mg of anhydrous tacrolimus per ml.

The oral absorption of tacrolimus is erratic and incomplete; absolute bio-availability is approximately 25%. Peak serum levels are seen 1 to 3 hours after an oral dose. Therapeutic trough blood concentrations have ranged from 5 to 20 ng/ml. Tacrolimus is extensively metabolized in the liver, with only small amounts of unchanged drug (2% or less) being recovered in the urine. The elimination half-life of tacrolimus is approximately 10 hours.

Tacrolimus suppresses both humoral (antibody) and cell-mediated immune responses. The compound is chemically distinct from cyclosporine but both agents elicit similar immunosuppressant effects. The immunosuppressive activity of tacrolimus is, however, more marked than that of cyclosporine.

10.5.2) PREPARATION : FOR IV USE:

Tacrolimus concentrate for injection must be diluted prior to IV infusion. For IV infusion, the concentrate will be prepared as per pharmacy standards. Preparation of the solution in polyethylene or glass containers allows storage for 24 hours beyond which unused solution should be discarded. A plasticized polyvinyl chloride (PVC) container should not be used because stability of the solution is decreased and polyoxyl 60 hydrogenated castor oil contained in the formulation may leach phthalates from PVC containers. Tacrolimus concentrate for injection and diluted solutions of the drug should be inspected visually for particulate

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matter and discoloration prior to administration whenever solution and container permit.

10.5.3) ADMINISTRATION:

Oral therapy should be started as soon as possible after transplantation and 8 to 12 hours after stopping intravenous therapy. In patients unable to tolerate oral therapy, the initial recommended intravenous dose is 0.03 to 0.05 mg/kg/day administered as a continuous infusion.

10.5.4) STORAGE & STABILITY:

Store tacrolimus capsules at controlled room temperature, 15-30°C (59-86°F) (Prod Info Prograf®, 1997). An extemporaneous suspension of tacrolimus with a final concentration of 0.5 milligrams/milliliter was stable for 56 days when it was stored at 24-26°C in glass or plastic amber prescription bottles.

10.5.5) TOXICITY:

In patients receiving tacrolimus, 5% to 47% experienced anemia, 8% to 32% experienced leukocytosis, and 14% to 24% experienced thrombocytopenia. Rare cases of microangiopathic hemolytic anemia have been reported. Mild to moderate hypertension was reported in 38% to 50% of patients receiving tacrolimus. Mild to moderate hypertension is a common adverse effect associated with tacrolimus therapy. Chest pain was reported in 19%. Antihypertensive therapy may be required. The most common adverse effects of tacrolimus have involved the central nervous system, and include headache (37% to 64%), tremors (48% to 56%), insomnia (32% to 64%), paresthesia (17% to 40%); and dizziness (19%). Tremor and headache may respond to a dosage reduction. Agitation, anxiety, confusion, seizures, depression, hallucinations, myoclonus, neuropathy, psychosis, incoordination, and abnormal dreams have been reported in 3% to 15% of tacrolimus-treated patients. Hyperkalemia (13% to 45%), hypokalemia (13% to 29%), hypophosphatemia (49%), and hypomagnesemia (16% to 48%) have been associated with tacrolimus therapy. In addition, hirsutism occurs only rarely with tacrolimus. Hyperuricemia has been reported in greater than 3% of tacrolimus-treated patients. Gastrointestinal adverse effects of

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tacrolimus have included nausea (32% to 46%), vomiting (14% to 29%), anorexia (7% to 34%), constipation (23% to 35%) and diarrhea (37% to 72%). Gingival hyperplasia observed in patients treated with cyclosporine has not been reported with tacrolimus therapy. Nephrotoxicity was reported in 36% to 40% and 52% of liver and kidney transplant patients receiving tacrolimus. Overt nephrotoxicity is usually seen early after transplantation and is characterized by an increased serum creatinine and a decrease in urine output. Hematuria has been reported in greater than 3% of tacrolimus-treated patients (Prod Info Prograf®, 1997). Abnormal liver function tests have been reported in 6% to 36% of patients receiving tacrolimus; ascites was reported in 7% to 27% of these patients.

Other miscellaneous effects that have occurred in clinical trials include pain (24% to 63%), fever (19% to 48%), asthenia (11% to 52%), back pain (17% to 30%), and peripheral edema (12% to 36%). The incidence of hyperglycemia is 17% and may require therapy with insulin. Other less frequently occurring effects (greater than 3%) include abscess, chills, peritonitis, and photosensitivity reactions. Anaphylaxis has been reported in a few patients receiving intravenous tacrolimus. Tacrolimus contains castor oil which has been associated with anaphylaxis in other drugs containing castor oil derivatives.

10.6) Mycophenolate (CellCept®):

CellCept (mycophenolate mofetil) is the 2-morpholinoethyl ester of mycophenolic acid (MPA), inosine monophosphate dehydrogenase (IMPDH) inhibitor.

10.6.1) Availability and administration:

CellCept is available for oral administration as capsules containing 250 mg of mycophenolate mofetil, tablets containing 500 mg of mycophenolate mofetil, and as a powder for oral suspension, which when constituted contains 200 mg/mL mycophenolate mofetil. Each vial of CellCept Intravenous contains the equivalent of 500 mg mycophenolate mofetil as the hydrochloride salt. Reconstitution and dilution with 5% Dextrose

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Injection USP yields a solution of mycophenolate mofetil, 6 mg/mL.

10.6.2) Clinical Pharmacology:

Mechanism of Action: Mycophenolate mofetil is rapidly absorbed following oral administration and hydrolyzed to form MPA, which is the active metabolite. MPA is a selective and uncompetitive, inhibitor of inosine monophosphate dehydrogenase (IMPDH), and therefore inhibits the de-novo pathway of guanosine nucleotide synthesis without incorporation into DNA. Because T- and B-lymphocytes are critically dependent for their proliferation on de-novo synthesis of purines, whereas other cell types can utilize salvage pathways, MPA has potent cytostatic effects on lymphocytes. MPA inhibits proliferative responses of T- and B-lymphocytes to both mitogenic and allospecific stimulation.

10.6.3) Pharmacokinetics:

Oral absorption of the drug is rapid and essentially complete. MPA is metabolized to form the phenolic glucuronide of MPA (MPAG) which is not pharmacologically active.

10.6.4) Metabolism:

Following oral and intravenous dosing, mycophenolate mofetil undergoes complete metabolism to MPA, the active metabolite. MPA is metabolized principally by glucuronyl transferase to form the phenolic glucuronide of MPA (MPAG) which is not pharmacologically active. In vivo, MPAG is converted to MPA via enterohepatic recirculation. Increased plasma concentrations of mycophenolate mofetil metabolites (MPA 50% increase and MPAG about a 3-fold to 6-fold increase) are observed in patients with renal insufficiency.

10.6.5) Excretion:

Orally administered radiolabeled mycophenolate mofetil resulted in complete recovery of the administered dose, with 93% of the administered dose recovered in the urine and 6% recovered in feces. Most (about 87%) of the administered dose is excreted in the urine as MPAG.

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10.6.6) Contraindications:

Allergic reactions to CellCept have been observed; therefore, CellCept is contraindicated in patients with a hypersensitivity to mycophenolate mofetil, mycophenolic acid or any component of the drug product. CellCept Intravenous is contraindicated in patients who are allergic to Polysorbate 80 (TWEEN).

10.6.7) Adverse effects:

The principal adverse reactions associated with the administration of CellCept include diarrhea, leukopenia, sepsis, vomiting, and there is evidence of a higher frequency of certain types of infections. Patients receiving immunosuppressive regimens involving combinations of drugs, including CellCept, as part of an immunosuppressive regimen are at increased risk of developing lymphomas and other malignancies, particularly of the skin. The risk appears to be related to the intensity and duration of immunosuppression rather than to the use of any specific agent. Oversuppression of the immune system can also increase susceptibility to infection, including opportunistic infections, fatal infections, and sepsis.

As usual for patients with increased risk for skin cancer, exposure to sunlight and UV light should be limited by wearing protective clothing and using a sunscreen. Lymphoproliferative disease or lymphoma developed in 0.4% to 1% of patients receiving CellCept (2 g or 3 g) with other immunosuppressive agents in controlled clinical trials of renal, cardiac, and hepatic transplant patients.

There are no adequate and well-controlled studies in pregnant women. However, as CellCept has been shown to have teratogenic effects in animals, it may cause fetal harm when administered to a pregnant woman. Therefore, CellCept should not be used in pregnant women unless the potential benefit justifies the potential risk to the fetus. It is recommended that CellCept therapy should not be initiated by the physician until a report of a negative pregnancy test has been obtained.

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In patients receiving CellCept (2 g or 3 g) in controlled studies for prevention of renal, cardiac or hepatic rejection, fatal infection/sepsis occurred in approximately 2% of renal and cardiac patients and in 5% of hepatic patients.

Severe neutropenia [absolute neutrophil count (ANC) $<0.5 \times 10^3$ / μ L] developed in up to 2.0% of renal, up to 2.8% of cardiac, and up to 3.6% of hepatic transplant patients receiving CellCept 3 g daily. If neutropenia develops (ANC $<1.3 \times 10^3$ / μ L), dosing with CellCept should be interrupted or the dose reduced, appropriate diagnostic tests performed, and the patient managed appropriately. Gastrointestinal bleeding (requiring hospitalization) has been observed in approximately 3% of renal transplant patients treated with CellCept 3g daily. Gastrointestinal perforations have rarely been observed.

10.7) Antithymocyte Globulin:

10.7.1) Class; Immune globulin.

10.7.2) Dosage, Adult (usual); 15-30mg/kg IV for a total of 14 days, however, we will be using 30mg/kg/day IV on days -3, -2, and -1.

10.7.3) The same dose will be used for pediatric and adult patients.

10.7.4) Administration: Reconstitute: dilute with NS, dextrose 5% and $\frac{1}{4}$ normal saline, or dextrose and $\frac{1}{2}$ normal saline. Adding ATGAM to dextrose is not recommended due to possible precipitation. In addition, highly acidic solutions can also, over time contribute to physical instability.

10.7.5) The bottle of solution should be inverted so that the undiluted drug does not contact the air inside. The concentration should not exceed 4mg/ml. Do not shake the diluted solution.

10.7.6) Indications: treatment of rejection in renal transplant patients.

10.7.7) Contraindications;

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- Weigh benefits to risk in patients with systemic reaction to test dose
- Continued use contraindicated with severe and unremitting thrombocytopenia or leukopenia, or systemic reactions such as generalized rash, tachycardia, dyspnea, hypotension, or anaphylaxis.

10.7.8) Adverse Effects;

- leukopenia
- N/V, diarrhea
- dizziness
- headache
- fever, chills
- chest pain
- acute renal failure
- apnea, dyspnea, ARDS

10.8) METHOTREXATE:

10.8.1) General:

Methotrexate is an antimetabolite which binds to dihydrofolic acid reductase, thereby preventing the reduction of folic acid to tetrahydrofolate. It interferes with DNA synthesis, repair, and cellular replication. Actively proliferating tissues are in general more sensitive to this effect.

10.8.2) Administration:

Methotrexate LPF® Sodium (methotrexate sodium injection), Isotonic Liquid, Preservative Free, for single use only, is available in 25 mg/ml, 2 ml, 4 ml, 8 ml, and 10 ml vials, containing 50 mg, 100 mg, 200 mg, and 250 mg of methotrexate respectively. If desired, the solution may be further diluted immediately prior to use with an appropriate sterile, preservative-free medium.

Methotrexate Sodium for Injection, Freeze Dried, Preservative Free, Low Sodium, for single use only, is

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available in 20 mg, 50 mg, and 1 gram vials, containing approximately 0.14, 0.33 and 7 meq of sodium respectively. Reconstitute immediately prior to use with an appropriate sterile, preservative-free medium.

Methotrexate Sodium Injection, Isotonic Liquid, Preservative Protected, is available in 25 mg/ml, 2 ml (50 mg), and 10 ml (250 mg) vials. The preservative formulation contains benzyl alcohol and must not be used for intrathecal or high dose therapy. If desired, the solution may be further diluted with a compatible medium.

10.8.3) Toxicities:

10.8.3.A) Hematologic: myelosuppression [leukopenia (nadir 7 days), thrombocytopenia, anemia].

10.8.3.B) Hepatic: acute (elevated transaminases) and chronic (fibrosis and cirrhosis) hepatic toxicity. Chronic toxicity has generally occurred after prolonged use (generally 2 years or more) and after a total dose of at least 1.5 grams.

10.8.3.C) Urogenital: severe nephropathy or renal failure, azotemia, cystitis, hematuria; defective oogenesis or spermatogenesis, transient oligospermia, menstrual dysfunction and vaginal discharge; infertility, abortion, fetal defects. Close attention to renal function including adequate hydration, and urine alkalization are essential for safe administration.

10.8.3.D) Gastrointestinal: gingivitis, pharyngitis, stomatitis, anorexia, nausea, vomiting, diarrhea, hematemesis, melena, gastrointestinal ulceration and bleeding, enteritis. Should be used with extreme caution in the presence of peptic ulcer disease or ulcerative colitis. Therapy may be discontinued if ulcerative

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stomatitis or other severe GI adverse reactions occur.

- 10.8.3.E) Pulmonary: interstitial pneumonitis deaths have been reported, and chronic interstitial obstructive pulmonary disease has occasionally occurred. Pulmonary symptoms or a nonspecific pneumonitis may be indicative of a potentially dangerous lesion and require interruption of treatment and careful investigation; infection needs to be excluded. This lesion can occur at all dosages.
- 10.8.3.F) Skin: erythematous rashes, pruritus, urticaria, photosensitivity, pigmentary changes, alopecia, ecchymosis, telangiectasia, acne, furunculosis.
- 10.8.3.G) Central Nervous System: headaches, drowsiness, blurred vision. There have been reports of leukoencephalopathy following intravenous administration of methotrexate to patients who have had craniospinal irradiation. Aphasia, hemiparesis, paresis, and convulsions have also occurred following administration of methotrexate. Following low doses, occasional patients have reported transient subtle cognitive dysfunction, mood alteration, or unusual cranial sensations.
- 10.8.3.H) Other: opportunistic infection, arthralgia/myalgia, loss of libido/impotence, diabetes, osteoporosis and sudden death. A few cases of anaphylactoid reactions have been reported.

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11.0) RESPONSE CRITERIA:

11.1) Lymphoma (IBMTR response criteria):

- 11.1.1) Complete Response (CR): Complete disappearance of all known disease for ≥ 4 weeks.
- 11.1.2) Complete Remission Undetermined (CRU); as above with the exception of persistent scan abnormalities of unknown significance.
- 11.1.3) Partial Remission (PR); $\geq 50\%$ reductions in greatest diameter of all sites of known disease and no new sites.
- 11.1.4) No Response/Progressive Diseases; $\leq 50\%$ reduction in greatest diameter of all sites of known disease or increase in size of known disease or new sites of disease.

11.2) Multiple Myeloma (IBMTR response criteria):

- 11.2.1) Complete Response (CR); Requires all of the following:
 - 1. Absence of monoclonal protein in urine and serum by electrophoresis and immunofixation for at least 6 weeks.
 - 2. $\leq 5\%$ plasma cells in marrow trephine biopsy (repeat biopsy in 6 weeks needed in non-secretory myeloma only).
 - 3. No increase in number or size of lytic bone lesion ; no new lesions.
- 11.2.2) Partial Response (PR); requires all of the following;
 - 1. $\geq 50\%$ reduction in serum paraprotein levels for at least 6 weeks.
 - 2. Reduction of urinary paraprotein to ≤ 200 mg/24hr or $\geq 90\%$ reduction in paraprotein excretion over 24 hours for at least 6 weeks.
 - 3. No increase in number or size of lytic bone lesions ; no new lesion.
 - 4. $\geq 50\%$ reduction in size of soft tissue plasmacytomas (by radiographs or exams).

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5. For non-secretory myeloma only, $\geq 50\%$ reduction in marrow plasma cells by marrow trephine biopsy for at least 6 weeks.

11.2.3) Minimal Response: Requires all of the following;

1. 25% –50% reduction in serum paraprotein level for at least 6 weeks.
2. 50%-90% reduction in urinary paraprotein but still ≥ 200 mg/24hrs for at least 6 weeks.
3. No increase in size or number of lytic lesions or extramedullary plasmacytomas.
4. For non-secretory myeloma only, 25-50% reduction in marrow plasma cells by bone marrow trephine biopsy for at least 6 weeks.

11.2.4) No response/Stable Disease (NR/SD); Not meeting criteria of either minimal response or progressive disease.

11.2.5) Progressive Disease: Includes any of the following;

1. $\geq 25\%$ increase in the level of serum monoclonal protein which must be an absolute increase of 5 gm/L above baseline and must be confirmed by at least 1 repeated evaluation.
2. $\geq 25\%$ increase in 24 hr urinary paraprotein from a minimum baseline amount of at least 500 mg/24 hrs on 2 occasions.
3. $\geq 25\%$ increase in bone marrow plasma cells on trephine biopsy from minimum baseline of 5% in non-secretory myeloma.
4. Increase in number and /or $\geq 25\%$ increase in size of extramedullary plasmacytomas.
5. New lytic bone lesions.
6. Development of hypercalcemia.

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11.3) Acute Leukemia:

Complete Remission (CR): <5% blasts in bone marrow biopsy, ANC \geq 1000/uL and platelets \geq 100,000/uL.

Cri: <5% blasts in bone marrow biopsy but ANC<1000/ul and/or platelets <100,000/ul.

CRc: fulfill definition for CR plus normalization of prior abnormal cytogenetics, N/A in patients with normal cytogenetics pre-transplant.

Stable disease: less than 25% change (+/-) in blast count from pre-transplant level.

Progression: >25% increase in blast count from pre-transplant level.

11.4) Response Criteria for Patients with Chronic Lymphocytic Leukemia & Prolymphocytic Leukemia:

11.4.1) Complete Response: requires all of the following for a period of at least two months from completion of therapy:

- Absence of lymphadenopathy on physical exam
- No hepatomegaly or splenomegaly on physical exam
- Absence of constitutional symptoms
- Normal CBC as exhibited by polymorphonuclear leukocytes \geq 1,500/ μ L, platelets \geq 100,000/ μ L, hemoglobin \geq 11.0 g/dL (untransfused), lymphocyte count \leq 5,000/ μ L.
- Bone marrow aspirate and biopsy must be normocellular for age with \leq 30% of nucleated cells being lymphocytes. Lymphoid nodules must be absent. If the marrow is hypocellular, a repeat determination should be performed in 2-3 months.
- Patients who fulfill the criteria for CR with the exception of a persistent cytopenia that is believed to be treatment related will be considered a partial response. Additionally, patients who fulfill the criteria of CR with the exception of having bone marrow lymphoid nodules will be considered a partial response.

11.4.2) Partial Response: Requires a \geq 50% decrease in peripheral lymphocyte count from pre-treatment value, \geq 50% reduction in lymphadenopathy,

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and/or $\geq 50\%$ reduction in splenomegaly/hepatomegaly for a period of at least two months from completion of therapy. Additionally, these patients must have one of the following:

- Polymorphonuclear leukocytes $\geq 1,500/\mu\text{L}$ or 50% improvement from pre-treatment value;
- Platelets $\geq 100,000/\mu\text{L}$ or 50% improvement from pre-treatment value.
- Hemoglobin ≥ 11.0 g/dL (untransfused) or 50% improvement from pre-treatment value.

11.4.3) Progressive Disease: Characterized by any one of the following events:

- $\geq 50\%$ increase in the products of at least two lymph nodes on two consecutive determinations two weeks apart (at least one lymph node must be ≥ 2 cm); appearance of new palpable lymph nodes.
- $\geq 50\%$ increase in the size of the liver and/or spleen as determined by measurement below the respective costal margin; appearance of palpable hepatomegaly or splenomegaly, which was not previously present.
- $\geq 50\%$ increase in the absolute number of circulating lymphocytes to at least $5,000/\mu\text{L}$.
- Transformation to a more aggressive histology (i.e., Richter's syndrome or prolymphocytic leukemia with $\geq 56\%$ prolymphocytes).
- Patients not fulfilling the above criteria for progressive disease but demonstrating a decrease in hemoglobin ≥ 2 g/dL, decrease $\geq 50\%$ in platelet or granulocyte count will not be rated as progressive disease because these may occur as both a consequence of therapy and of underlying CLL. A bone marrow biopsy in such settings is strongly encouraged.

11.4.4) Stable Disease: Patients who do not fulfill the criteria for complete or partial response as defined above but do not exhibit progressive disease will be considered as having stable disease.

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11.5) Aplastic Anemia:

Response:

Neutrophils: ≥ 500 cells/mcl of colony stimulating factors.

Platelets: $\geq 50K$ untransfused
 $\geq 100K$ untransfused

Hgb: ≥ 8 untransfused

11.6) Fanconi anemia: Engraftment and negative chromosomal breakage on DEB testing will be used to evaluate response for Fanconi's anemia.

Response:

Neutrophils: ≥ 500 cells/mcl of colony stimulating factors.

Platelets: $\geq 50K$ untransfused
 $\geq 100K$ untransfused

Hgb: ≥ 8 untransfused

12.0) REMOVAL OF PATIENTS FROM PROTOCOL AND ADVERSE EVENT REPORTING (AER):

12.1) Removal from protocol:

If the constraints of this protocol are detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the patient will be removed from the protocol. In this event the reason for withdrawal will be documented and the patient will be followed for survival. Any patient with rapid disease progression will be removed from the protocol. Document details, including tumor measurements on flow sheets and follow the patient for survival and secondary malignancies.

12.2) Toxicity Reporting:

12.2.1) Expected toxicity: (recorded in protocol consent form or manufacturer's literature).

Within 10 days of occurrence, written reports should be submitted to the IRB and the protocol chairman in the following circumstances (Bearman toxicity).

- Any fatal toxicity.
- Any non-hematologic grade 3 toxicity.

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- Grade 3-4 acute GvHD.

- 12.2.2) Unexpected toxicity: All instances of Bearman grade 3-4 toxicity will be reported to the IRB and the protocol chairman immediately by telephone and followed by a written report.
- Failure to engraft (patient alive on day 45 with no ANC recovery)
 - Graft Failure (patient had initial ANC recovery but subsequently had a decline in ANC that required additional stem cell support)

13.0) STATISTICAL CONSIDERATIONS:

13.1) Power Computations

Sample size calculation is based on the primary endpoint: toxicity, defined as day 100 treatment related mortality (TRM). The null hypothesis is that the TRM is $< 25\%$ vs. the alternative hypothesis that the TRM is $> 40\%$. A two-stage study design will be used in which a total of 60 patients with hematologic malignancies and disorders will be accrued. If more than 20 patients experience TRM, the null hypothesis will be rejected and the treatment is not considered worthy of further study.

13.2) Early Stopping Rules

Assessment of the day 100 TRM rate is significantly higher than 40%. At stage 1 with accrual of 30 patients, if 13 or more patients die of TRM, the study will be closed. Otherwise accrual will continue until the target of 60 patients or if 20 or more patients die of TRM.

The decision rules of this study design yield a type I error rate = 0.0933 and power = 0.8709.

13.3) Secondary End points

The secondary endpoint is disease response rate. Although response rate will be assessed, no statistical comparisons will be

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performed because we expect too heterogeneous a population of diseases to compare.

13.4) October 2003 Amendment

Due to slow accrual, the study design has been changed as follows. The null and research hypotheses, denoted H_0 and H_1 , respectively, continue to be that the day 100 treatment related mortality (TRM) = 25% versus TRM > 25%. Let S_1 and S_2 be the numbers of evaluable patients accrued at stages 1 and 2 who die due to treatment. Then, the following new two-stage design replaces the original two-stage design described in Section 13.2.

At stage 1, accrue 17 evaluable patients. If $S_1 \geq 10$, the study will be closed to accrual and it will be concluded that the day 100 TRM exceeds 25%. That is, if 10 or more patients among the first 17 evaluable patients experience treatment related death on or before day 100, it will be concluded that the day 100 TRM exceeds 25%. If $S_1 \leq 9$ continue to stage 2.

At stage 2, accrue 16 more evaluable patients. If $S_1 + S_2 \geq 12$, conclude that the day 100 TRM exceeds 25%. Otherwise, there is insufficient evidence to draw this conclusion.

With this design, the probability is 0.099 of erroneously concluding H_1 and the probability is 0.880 that H_1 will be correctly concluded if the true rate is 45%. That is, the significance level of the test is 0.099 and 0.880 is the power of the test when 45% the true rate.

This design is optimal in the following sense. The stage 1 sample size, 17, is as small as possible. The stage 2 sample size, 16, is as small as possible subject to the constraint that it is bounded above by the stage 1 sample size. The significance level of the test is bounded above by 0.10, and the power of the test when the day 100 TRM is 45% is bounded below by 0.88.

Observing disease response rate continues to be a secondary objective. No statistical comparisons among disease-type response rates will be made because of the heterogeneity of diseases.

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Appendix No. I

Bearman* criteria for toxicity grading

	Grade I	Grade II	Grade III
Cardiac	Mild EKG abnormality, not requiring medical intervention; or noted heart enlargement on CXR with no clinical symptoms	Moderate EKG abnormalities requiring and responding to medical intervention; or requiring continuous monitoring without treatment; or congestive heart failure responsive to digitalis or diuretics	Severe EKG abnormalities with no or only partial response to medical intervention; or heart failure with no or only minor response to medical intervention; or decrease in voltage by more than 50%
Bladder	Macroscopic hematuria after 2 days from last chemotherapy dose with no subjective symptoms of cystitis and not caused by infection	Macroscopic hematuria after 7 days from last chemotherapy dose not caused by infection; or hematuria after 2 days with subjective symptoms of cystitis not caused by infection	Hemorrhagic cystitis with frank blood, necessitating invasive local intervention with installation of sclerosing agents, nephrostomy or other surgical procedure
Renal	Increase in creatinine up to twice the baseline value (usually the last recorded before start of conditioning)	Increase in creatinine above twice baseline but not requiring dialysis	Requirement of dialysis
Pulmonary	Dyspnea without CXR changes not caused by infection or congestive heart failure; or CXR showing isolated infiltrate or mild interstitial changes without symptoms not caused by infection or congestive heart failure	CXR with extensive localized infiltrate or moderate interstitial changes combined with dyspnea and not caused by infection or CHF; or decrease of PO ₂ (>10% from baseline) but not requiring mechanical ventilation or > 50% O ₂ on mask and not caused by infection or CHF	Interstitial changes requiring mechanical ventilatory support or >50% oxygen on mask and not caused by infection or CHF
Hepatic	Mild hepatic dysfunction with bili > 2.0 mg% but < 6.0 mg %; or weight gain > 2.5 % and < 5 % from baseline of noncardiac origin; or SGOT increase more than 2-fold but less than 5-fold from lowest preconditioning	Moderate hepatic dysfunction with bili >6 mg% < 20 mg %; or SGOT increase with > 5-fold from preconditioning; or clinical ascites or image documented ascites > 100ml; or weight gain > 5% from baseline of noncardiac origin	Severe hepatic dysfunction with bili > 20 mg %; or hepatic encephalopathy; or ascites compromising respiratory function
CNS	Somnolence but the patient easily arousable and oriented after arousal	Somnolence with confusion after arousal; or other new objective CNS symptoms with no loss of consciousness not more easily explained by other medication, bleeding, or CNS infection	Seizures or coma not explained by other medication, CNS infection, or bleeding
Stomatitis	Pain and/or ulceration not requiring a continuous IV narcotic drug	Pain and/or ulceration requiring a continuous IV narcotic drug	Severe ulceration and/or mucositis requiring preventive intubation; or resulting in documented aspiration pneumonia with or without intubation
GI	Watery stools > 500 ml but < 2,000 ml every day not related to infection	Watery stools > 2,000 ml every day not related to infection; or macroscopic hemorrhagic stools with no effect on cardiovascular status not caused by infection; or subileus not related to infection	Ileus requiring nasogastric suction and/or surgery and not related to infection; or hemorrhagic enterocolitis affecting cardiovascular status and requiring transfusion

NOTE: Grade IV regimen-related toxicity is defined as fatal toxicity

*Bearman SI et al. Regimen Related Toxicity in Patients Undergoing Bone Marrow Transplantation. JCO 1988, 6(10); 1562-15

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Appendix No. II
Calculation of adjusted ideal body weight (36)

To calculate ideal body weight for men

$$50 + [0.91 \times (\text{height in cm} - 152)]$$

OR

$$50 + [2.3 \times (\text{height in inches} - 60)]$$

To calculate ideal body weight for women;

$$45 + [0.91 \times (\text{height in cm} - 152)]$$

OR

$$45 + [2.3 \times (\text{height in inches} - 60)]$$

Adjusted ideal body weight

$$\text{Ideal body weight} + [0.25 \times (\text{actual body weight} - \text{ideal body weight})]$$

The calculation of ideal body weight is referenced in the package insert of Busulfex (busulfan) Injection.

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Appendix No. III CRITERIA FOR ACUTE GRAFT-VS-HOST DISEASE

Clinical staging of acute graft-vs.-host disease according to organ involvement

STAGE	SKIN	LIVER	INTESTINAL TRACT
0	No rash	Bilirubin < 2.0 mg/dL < 34µmol/L	Diarrhea 500 ml/day
+	Maculopapular rash <25% of body surface	Bilirubin 2-2.9 mg/dL 34-50 µmol/L	Diarrhea 500-1000 ml/day Peds: ≥30 ml/kg, < 60ml/kg
++	Maculopapular rash 25-50% of body surface	Bilirubin 3.0-6.0 mg/dL 51-102 µmol/L	Diarrhea 1000-1500 ml/day Peds: ≥ 60 ml/kg, < 90 ml/kg
+++	> 50% body surface	Bilirubin 6.1-15 mg/dL 103-255 µmol/L	Diarrhea 1500 ml/day Peds: ≥90 ml/kg
++++	Generalized erythroderma with bullous formation and desquamation	Bilirubin > 15 mg/dL > 255 µmol/L	Severe abdominal pain with or without ileus

Clinical grading of severity of acute graft-vs-host disease

GRADE	DEGREE OF ORGAN INVOLVEMENT
I	+ to ++skin rash; no gut involvement; no liver involvement; no decrease in clinical performance
II	+ to +++ skin rash; + gut involvement or + liver involvement (or both); mild decrease in clinical performance
III	++ to +++ skin rash; ++ to +++ gut involvement or ++ to ++++ liver involvement (or both) marked decrease in clinical performance
IV	Similar to Grade II with ++ to ++++ organ involvement and extreme decrease in clinical performance

Source: Thomas et al, N Engl. J Med. 1975; 292, 832

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Appendix No. IV
Clinical Grading of Chronic GVHD

Limited Chronic GVHD:

1. Localized skin involvement,
and/or
2. Hepatic dysfunction due to chronic GVHD.

Extensive Chronic GVHD:

1. Generalized skin involvement, or
2. Localized skin involvement and/or hepatic dysfunction due to chronic GVHD

Plus

- 3a. Liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis, or
- 3b. Involvement of eye (Schirmer's test with less than 5 mm wetting), or
- 3c. Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy, or
- 3d. Involvement of any other target organ.

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Appendix No. V

Stem Cell Infusion:

Day 0 is the day on which the stem cells are infused. The procedure of infusing stem cell products may be performed by the physician or the BMT Coordinator. Stem cells are to remain sterile throughout the infusion process. All patients require continuous pulse oximetry monitoring during the procedure, with oxygen equipment available in the patient's room. All patients will have vital signs recorded before the procedure and at timed intervals during and after stem cell infusion. Emergency drugs, such as benadryl, epinephrine and corticosteroids will be available for use in appropriate doses. No other blood products should be given on the day of transplant, especially within 8 hours of planned infusion time. Stem cells must be infused without the use of a blood filter. No more than 480 ml of stem cells may be infused at one time. Patients will be pre-medicated according to RPCI Standard Operating Procedure for Stem Cell reinfusion.

Cord Blood Stem Cell Preparation and Reinfusion (37).

Cryopreserved units of cord blood stem cells will be shipped to Roswell Park in liquid nitrogen in vapor phase and stored in liquid nitrogen prior to patients receiving their preparative regimen. Subsequently the unit will be thawed in the laboratory and washed with 10 % Dextran 40 and 5% human albumin before infusion. A nucleated cell count, CD34⁺ count, ABO and Rh typing, test of cell viability, bacterial and fungal cultures and assay for

hematopoietic progenitors will be performed on a sample from each thawed unit if there are more than adequate cells for transplant. If there is a limited amount of cord blood, then samples will be sent for bacterial and fungal cultures and cell viability only. The cord blood product is then infused intravenously into the patient. Cord blood units will not be T-cell depleted, volume reduced or depleted of red cells.

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Appendix No. VI
Karnofsky Performance Status (KPS)

KPS	DEFINITION
100	Normal; no complaints; no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some sign or symptoms of disease
70	Cares for self; unable to carry on normal activity or do active work
60	Requires occasional assistance, but is able to care for most personal needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled; hospitalization is indicated, although death not imminent
20	Very sick; hospitalization necessary; active support treatment is necessary
10	Moribund; fatal processes progressing rapidly
0	Dead

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Appendix No. VII

Child-Pugh Classification of Liver Failure

Measurement	Points Scored 1	Points Scored 2	Points Scored 3
Encephalopathy	None	1 and 2	3 and 4
Ascites	None	Slight	Moderate
Bilirubin (mg/100 ml)	1.0 – 2.0	2.0 – 3.0	> 3.0
Albumin (gm/ 100 ml)	3.5	2.8 - 3.5	< 2.8
Prothrombin time (sec. prolonged)	1 - 4	4 - 6	>6
For PBC, bilirubin (mg/100 ml)	1 - 4	4 - 10	>10

Child - Pugh Classification:

A = 1 – 6, **B** = 7 – 9, **C** = 10-15

Protocol Number: RP 01-05

Appendix No. VIII

Tests/Observations*	Pre-Rx	Post-Rx
History and physical exam	X	--
Signed Informed Consent	X	--
Ht/Wt/IBW/BSA	X	--
Ht/Wt/IBW/BSA	X	--
Serology (HIV, Hepatitis, CMV)	X	--
HLA-A, B typing	X	--
Cr Clearance	X	--
PFT's/ DLco	X	--
PFT's/ DLco	X	--
ABG/Exercise Study (if indicated)	X	--
MUGA	X	--
EKG	X	--
Quantitative Immunoglobulins	X	X ^Ω
BM aspirate, biopsy		day 30-40, day 100-120 and then as indicated in section 8.6.3
Peripheral blood for chimerism	X	day 30-40, 60-70 and then as indicated in section 8.6.3
Molecular and cytogenetics analysis	X	day 30-40,100-120 and then as clinically indicated
CBC/Plt	X	q day till PMN≥500, Plt≥20K, and then as clinically indicated
Urinalysis	X	--
PT/PTT	X	--
P22, LDH	X	q wk x 4 and then as indicated
Chest X-ray	X	as clinically indicated
CT Scan ^Ψ	X	q wk x 4 and then as clinically indicated
Karnofsky Performance Status	X	q wk x 4 and then as clinically indicated
Toxicity (Bearman)	--	wkly x 4 and then day 100 & then as clinically indicated
Acute GVHD	--	q wk till day +100
Chronic GVHD	--	twice a year

- * Day 0 is day of stem cell transplant; studies to be obtained as close to indicated time as possible. Intervals shown are the minimum requirement.
- Ω Post transplant SPEP, IFE, Quantitative immunoglobulins, serum beta-2 microglobulin and Urine protein electrophoresis will be done only for patients with multiple myeloma on day 100-120 and then as clinically indicated.
- Σ BM aspirate and biopsy will be performed for patients with leukemias only. Patients with other malignancies will have BM aspirate and biopsy only if bone marrow was involved previously. Chimerism study will be done on each specimen. Cytogenetics will be done if it was abnormal previously.
- Φ In pts who receive DLI, a final sample should be collected day + 100 post DLI completion
- Ψ For patients with lymphoma only. Also palpable tumor measurement should be recorded for lymphoma patients.