NON-MYELOABLATIVE CHEMOTHERAPY FOLLOWED BY HLA-MATCHED RELATED ALLOGENEIC STEM CELL TRANSPLANTATION FOR HEMATOLOGIC MALIGNANCIES

Protocol Number: D0345

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SCHEMA

Informed Consent and Pre-study Evaluations

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Pre-Transplant Treatment Regimen

Fludarabine	25mg/m^2	IV	Once daily over 30 minutes on days -6 through -2
Cyclophosphamide	1mg/m^2	IV	Once daily over 2 hours on days -3 and -2
Mesna	20% of cyclophosphamide dose	IV	15 minutes prior to cyclophosphamide, 4 and 8 hours following cyclophosphamide
Cyclosporine	3mg/kg if IV 5mg/kg if PO	IV/PO	Once daily starting on day -4 if IV, Twice daily starting on day -4 if PO

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Transplant (Day 0)

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Post-Transplant Treatment Regimen

Methotrexate	10mg/m^2	IV	Days +1, +3 and +6

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Post Transplant Follow-Up

Days +30 & +60 (both $\pm$3 days), Days +100, +180, +365 (all $\pm$15 days)

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Short Term Follow-up

(Every three months for two years following 1 year visit, then every year for 5 years or until death or relapse, whichever occurs first)

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Long Term Follow-up **(Yearly survival check)**

1.0 OBJECTIVES

Primary Objectives

- To evaluate engraftment by measuring donor-recipient chimerism in lymphoid and myeloid lineages
- To investigate the effect of donor lymphocyte infusion on donor-host chimerism
- To determine the incidence and severity of acute and chronic graft-versus-host disease
- To assess the following:
    - treatment-related mortality
    - time to progression
    - disease-free survival
    - overall survival
    - regimen-related toxicity

Secondary Objective

- To evaluate disease response rate as defined in section 12.3

2.0 INTRODUCTION

2.1 Allogeneic Bone Marrow Transplantation Background

Allogeneic bone marrow transplantation (BMT) became feasible in the 1960s after elucidation of the Human Leukocyte Antigen (HLA) complex. Since then, the therapy has evolved into an effective treatment for many hematologic disorders [1]. Otherwise incurable malignancies are frequently cured by this approach, with the likelihood of cure ranging from 10% to 85%, depending on the disease and the disease status. The treatment strategy incorporates very large doses of chemotherapy and often radiation to eliminate cancer cells and to immunosuppress the recipient to allow the engraftment of donor cells. Donor cells give rise to hematopoiesis within two to three weeks, rescuing the patient from the effects of high dose therapy. In the ideal situation, immune recovery and recipient-specific tolerance occurs over the following 6-18 months, and the patient is cured of their underlying malignancy, off immunosuppression, with a functionally intact donor-derived immune system. However, complications are common and include fatal organ damage from the effects of high dose chemotherapy, infection, hemorrhage, and, in particular, graft-versus-host disease (GvHD). A realistic estimate of transplant-related mortality in the standard HLA-matched sibling setting is approximately 25%. The risk of treatment–related mortality limits the success and certainly precludes its use in older patients. Thus, new strategies in transplantation are needed.

2.2 Shifting emphasis in BMT toward adoptive immunotherapy

With the growing understanding that much of the curative potential of allogeneic bone marrow or stem cell transplant (SCT) is from an immune anti-tumor effect of donor cells, known as *graft-versus-leukemia* (GvL) or *graft-versus-tumor* (GvT) [2-5], a new strategy is being employed that shifts the emphasis from high-dose chemo-radiotherapy to donor-derived, immune-mediated anti-tumor therapy. In this approach, patients receive preparative regimens that, while having some anti-tumor activity, are mainly designed to be immunosuppressive enough to allow engraftment of donor stem cells and lymphocytes [6-11]. Engrafted lymphocytes then mediate a GvL effect; if the GvL effect of the initial transplant is not sufficient, then additional lymphocytes may be infused (achievement of engraftment allows additional lymphocytes to "take" in the recipient without requiring any additional conditioning of the recipient). The lower intensity of the preparative regimen lessens the overall toxicity by minimizing the doses of chemo-radiotherapy. In addition, less intensive preparative regimens may be associated with less GvHD, as much evidence suggests that high-dose therapy contributes to the syndrome of GvHD by causing tissue damage, leading to a cytokine milieu which enhances activation of graft-versus-host (GvH) effector cells [12]. Thus, such an approach may allow the safer use of allogeneic transplants in standard populations and may allow extension of allogeneic transplantation to patients who could not receive standard (myeloablative) transplants because of age or co-morbidities. This protocol investigates a non-myeloablative transplant approach, using fludarabine and cyclophosphamide, to allow engraftment of allogeneic cells, which may then mediate anti-tumor effects.

2.3 Graft-vs-Leukemia

In the past, it was thought that allogeneic transplant cured malignancies simply by allowing the delivery of high doses of chemotherapy and radiation. However, it has become increasingly clear over the past few years that much of the curative potential of allogeneic SCT derives from an anti-tumor effect mediated by donor-immune cells. This GvL phenomenon may account for as much as half of the curative potential of the allogeneic SCT process.

Numerous animal studies since the mid-1950s have documented an anti-leukemic effect of allogeneic bone marrow transplantation [2,3]. Observations in humans have strongly suggested the existence of a GvL effect in human allogeneic BMT [4,5]: relapse rates of syngeneic BMT recipients are higher than in allogeneic BMT recipients; allogeneic recipients who do not develop GvHD relapse more frequently than allogeneic recipients who do develop GvHD; and recipients of T-cell depleted BMT relapse more frequently than recipients of non-T-cell depleted BMT. In addition, GvL has been purposefully induced simply by withdrawal of immune suppression (e.g., cyclosporine, steroids), which presumably allows the expansion of allogeneic donor lymphocytes with anti-tumor reactivity; remissions following this immunologic maneuver have been reported in AML, CML and NHL.

Based on the potential power of the GvL effect and its presumed mediation by donor leukocytes (T cells and/or natural killer [NK] cells), several groups have infused leukocytes obtained from the original bone marrow donor into patients with relapsed malignancy after

allogeneic BMT [16-26]. Most patients treated with donor leukocyte infusions (DLI) have had chronic myelogenous leukemia; durable complete remissions have been reported in 50-70% of these patients. Ongoing remissions have also been reported in patients with AML, ALL, multiple myeloma, NHL and CLL.

DLIs are often associated with GvHD. In one series, acute and chronic GvHD occurred in 60% of patients [23]; however, GvHD was closely associated with disease response ($p<.00001$). Several observations have suggested that it may be possible to separate GvL from GvHD in some cases. Occasional patients receiving typical doses of DLI (10^8 T cells/kg) have been observed who had complete responses but did not develop GvHD [22, 23]. A higher percentage of patients receiving lower doses of donor leukocytes (10^7 T cells/kg) had complete responses but no GvHD [27, 28]. The incidence and severity of GvHD (both acute and chronic) also appears to be significantly lower when escalating doses of DLI are used, with similar or improved GvL effect [27, 28]. Two groups have administered DLI depleted of $CD8^+$ cells and observed GvL without GvHD in the majority of patients [29, 30].

Response to DLI in patients with the following diseases provides definitive evidence for GvL in a percentage of patients.

2.4 Chronic Myelogenous Leukemia

The GvL effect is most pronounced in CML. An allogeneic GvL effect in CML is supported by the following evidence:

a. A lower risk of relapse in recipients who develop GvHD after allogeneic BMT (relative risk 0.24 for patients with both acute and chronic GvHD, p= .03) [5].
b. A trend towards increased risk of relapse in syngeneic transplant recipients (relative risk 2.95, p=.08)
c. An increased risk of relapse in recipients of T cell depleted allografts (relative risk 5.14, p= .0001);
d. Cyclosporine withdrawal-induced remission in CML patients in relapse after allogeneic BMT [14];
e. Complete remission following treatment with DLI in 50-75% of patients with CML in relapse after allogeneic BMT [22, 23].

In addition, there is evidence that supports a graft-vs.-tumor effect in other hematologic diseases as noted below:

2.5 Acute Myelogenous Leukemia and Acute Lymphoblastic Leukemia

a. A lower risk of relapse in recipients who develop GvHD after allogeneic BMT (relative risk 0.34 for patients with both acute and chronic GvHD, p= .0003) [5];
b. An increased risk of relapse in syngeneic transplant recipients (relative risk 2.58, p= .008) [5];
c. Cyclosporine withdrawal-induced remission in AML (>ALL) patients in relapse after allogeneic BMT [5];

d. Complete remission to treatment with DLI alone in patients in cytogenetic-only relapse after allogeneic BMT (4 of 9 patients in one series) (Collins, RH et al. unpublished observations);
e. Ongoing disease-free survival in 20% of patients in hematologic relapse after allogeneic BMT who are treated with combined DLI and chemotherapy (Collins, RH et al. manuscript in preparation)

2.6 Multiple Myeloma

a. A lower risk of relapse in patients treated with allogeneic as opposed to autologous transplantation (an alternative explanation is re-infusion of tumor cells in autologous transplants) [26];
b. Clear-cut complete remissions to DLI in patients in relapse after allogeneic BMT [25, 26].

2.7 Non-Hodgkin's Lymphoma

a. A lower risk of relapse in patients treated with allogeneic as opposed to autologous transplantation. In two large studies, patients with lymphoma were assigned to allogeneic versus autologous transplants based on the availability of a matched sibling donor [31, 32]. Relapse rates were significantly reduced in recipients of allografts;
b. Withdrawal of immunosuppression-induced remission (associated with GvHD in relapsed NHL after allogeneic BMT) [16].
c. Clear-cut disease response to DLI [16, 23]. Overall, the number of NHL patients treated with DLI is low, making an estimate of response rate difficult at this time.

2.8 Myelodysplastic syndrome (MDS)

A graft-vs.-tumor effect in MDS is supported by complete responses to DLI in patients with relapsed disease after allogeneic BMT (4 of 10 patients; Collins, RH et al. unpublished observations). However, more patients need to be treated to allow definitive assessment of response to this maneuver.

2.9 Chronic Lymphocytic Leukemia (CLL)

a. Relapse rates are significantly reduced in CLL allografts versus autografts [33] (again, reinfusion of tumor cells with autografts is an alternative explanation);
b. Clear-cut tumor responses have been observed in patients with residual or relapsed disease treated with DLI [24]. Not enough patients have yet been reported to allow estimation of response rate.

2.10 Hodgkin's Disease

A graft-versus-tumor effect is supported mainly by the reduced relapse rate in allogeneic versus autologous or syngeneic transplant recipients [34]. To date, very few DLIs have been reported in Hodgkin's disease; 1 of 5 patients in a series has responded (Collins et al, unpublished data). In addition, Porter et al. have reported two patients with relapsed Hodgkin's disease after allogeneic BMT who achieved responses, including a complete response after DLI [35].

2.11 Allogeneic Peripheral Blood Stem Cell (PBSC) Transplants

Stem cells for allogeneic transplantation may be obtained from either the bone marrow or the blood. Peripheral blood stem cells are obtained by apheresis after the donor has received granulocyte colony stimulating factor (G-CSF) for several days to mobilize stem cells from the marrow to the blood [36]. The more immunologically mature peripheral blood stem cells and higher number of T cells in this pheresis collection increases the rate and overall success of engraftment relative to bone marrow-derived stem cells, particularly with regard to the platelet cell line. Despite these immunologic features, peripheral blood stem cell transplants do not result in a higher incidence of acute GvHD, though retrospective case-control studies do suggest that the incidence of chronic GvHD may be higher.

2.12 Non-Myeloablative Preparative Regimens

As described in detail above, allogeneic BMT has curative potential in several hematologic malignancies. Due to the toxicity of standard BMT procedures, however, older patients or younger patients with co-morbid illnesses or organ dysfunction cannot be offered this potentially curative therapy. Since the curative potential of allogeneic BMT may be derived from the anti-tumor effect of donor immune cells, it is possible to harness this effect by discontinuing immunosuppression after donor cell engraftment, or by infusing additional immune cells without immunosuppression. The non-myeloablative therapy is immunosuppressive enough to allow donor cell engraftment [37] [6-11]. Allogeneic peripheral blood stem cells are infused following therapy, and once engrafted these donor immune cells may eliminate residual tumor cells via the GVL effect. If the malignancy persists, then the GvT effect may be enhanced by stopping immunosuppression or by infusing additional donor immune cells (DLI). Attainment of mixed (donor/recipient) chimerism after engraftment serves as the necessary platform for subsequent DLI.

We will use this non-myeloablative regimen to treat hematologic malignancies. The design of the study incorporates some of the following key features:

1. Immunosuppression without myeloablation: Patients will receive chemotherapy sufficient to allow donor lymphohematopoietic engraftment without complete marrow ablation. If the graft is rejected, the patient will reconstitute autologous marrow and survive. We will use a combination of two agents with known immunosuppressive activity in BMT: Cyclophosphamide, which produces transient pancytopenia, and Fludarabine, which causes less cytopenia but equivalent immunosuppression.
2. PBSC transplant: An unmanipulated peripheral blood stem cell collection from a G-CSF stimulated HLA-matched related donor should improve the chance of engraftment because of the high stem cell dose (>5 x 10^6/kg $CD34^+$ cells) and the presence of donor lymphocytes. Low intensity preparative regimens have a low incidence of severe GvHD. To further reduce the risk of GvHD, patients will receive cyclosporine (CSA) for the first 100 days after the transplant.

3. Use of donor lymphocyte infusions (DLI) for patients with less than full donor chimerism and/or residual disease to optimize post-transplant engraftment and anti-tumor effect.

3.0 STUDY DESIGN

3.1 Collection of Donor Cells

Collection of donor cells will be performed per the institution's standard practice. The goal of leukapheresis will be $\geq$ 5 x 10^6 CD34+cells/kg. In the unlikely event of a suboptimal PBSC collection, the donor will undergo a bone marrow harvest (non-mobilized) following a delay of at least 3 days.

3.2 Patient Step I - Peripheral Blood Stem Cell (PBSC) Transplant (Refer to Section 7.2 for details)

Participants will receive fludarabine 25mg/m^2/d IV over 30 minutes on days -6 to -2, followed by cyclophosphamide 1g/m^2/d IV on days -3 and -2. This will be followed by allogeneic stem cell infusion 48 hours later. The allogeneic HSC graft will aim to deliver greater than 3.5 x 10^6 CD34+ cells/kg of recipient weight, with a minimum of 2 x 10^6 $CD34^+$ cells/kg of recipient weight.

3.3 Patient Step II - Donor Lymphocyte Infusion (DLI) and Adjustment of Immunosuppression (Refer to Sections 7.4 and 7.5 for details)

Cyclosporine (CSA) and methotrexate (MTX) will be used for GvHD prophylaxis with target serum CSA levels of 200-400 ng/ml.

Refer to the Treatment Section (Section 7.5) for adjustment and tapering of immunosuppression and for Donor Lymphocyte Infusions.

4.0 ELIGIBILITY CRITERIA

4.1 Inclusion Criteria

4.1.1 Age: 18-75 years

4.1.2 Diseases

a. Chronic myelogenous leukemia (CML)
   - First chronic phase or later
   - Accelerated phase

b. Acute myelogenous or lymphoblastic leukemia (AML or ALL)
   - Second or subsequent remission
   - First remission with poor risk features, including, but not limited to:
     For AML- complex chromosome karyotype, abnormalities of chromosome 5 or 7, 12p-, 13+, 8+, t(9;22), t(11;23)
     For ALL- t(9;22), t(4;11), t(1;19), myeloid antigen coexpression
   - Patients who have failed an autologous PBSC transplant

c. Myelodysplastic syndrome (MDS)

d. Multiple myeloma - high risk myeloma (poor responders, relapse after autologous PBSCT, chromosome 13 abnormalities)

e. Hodgkin's disease

- Primary refractory disease
- Relapsed disease (first relapse or later)
- Patients who have failed an autologous PBSC transplant

f. Non-Hodgkin's lymphoma

Low grade (by Working Formulation)

- Relapsed, progressive disease after initial chemotherapy
- Primary refractory disease or failure to respond (>PR) to initial chemotherapy
- Patients who have failed an autologous PBSC transplant

Intermediate grade (by Working Formulation)

- Relapsed disease
- Primary refractory disease or failure to respond (>PR) to initial chemo
- Mantle cell lymphoma
- Patients who have failed an autologous PBSC transplant

g. Chronic lymphocytic leukemia (CLL)

- Patients newly diagnosed with poor prognostic factors, including CD38 expression, Chromosome 11 or 17 abn
- T-CLL/PLL
- Relapsed or progressive disase, or refractory after Fludarabine
- Patients who have failed an autologous PBSC transplant

4.1.3 Donor Availability: Six of six matched HLA A, B and DR identical sibling (or parent or child) or 5/6 related donor with single mismatch at Class I antigen (A or B)

4.1.4 Karnofsky performance status of $\geq$70%

4.1.5 Serum bilirubin $\leq$2x upper limit of normal; transaminases <3x normal (unless due to disease)

4.1.6 24 hr urine creat clearance of $\geq$ 40 ml/min.

4.1.7 DLCO ≥50% predicted

4.1.8 Left ventricular ejection fraction ≥35%

4.1.9 Voluntary written informed consent must be given before performance of any study-related procedure, with the understanding that consent may be withdrawn by the participant at any time without prejudice to future medical care. The participant must have the ability to understand and the willingness to sign a written informed consent document.

4.2 Exclusion Criteria

4.2.1 Major organ dysfunction

4.2.2 Pregnant or lactating female

4.2.3 Active infection

4.2.4 Psychological problems that preclude compliance and completion of the clinical trial

4.2.5 Any other condition, that in the judgement of the investigator, affects participant safety or overall participation

5.0 STAGING WORKUP AND PATIENT CLINICAL EVALUATION

5.1 Pre-study evaluation

***Based on clinical considerations, each patient will begin treatment on this protocol within a clinically relevant timeframe following completion of the pre-transplant evaluation**

5.1.1 HLA typing (molecular)
5.1.2 Patholologic evaluation confirming the diagnosis.
5.1.3 Disease staging (varies by disease- see appendix)
5.1.4 Serologic testing (IgM and IgG): CMV; HSV; VZV; toxoplasmosis
5.1.5 Antibody screen for Hep A, HBV, HCV, HIV, HTLV I/II, VDRL (or RPR)
5.1.6 CBC with differential, PT/PTT, CMP
5.1.7 ABO Rh testing
5.1.8 PSA (males over age 40 or with 1st degree family history of prostate cancer)
5.1.9 Serum pregnancy test (female with child-bearing potential; if clinically appropriate)
5.1.10 Mammogram and PAP smear (females) – within the past 12 months or as clinically indicated
5.1.11 Pulmonary function tests with DLCO
5.1.12 Cardiac function: EKG, MUGA scan (or ECHO)
5.1.13 24 hr urine for creatinine clearance as clinically indicated
5.1.14 Dental evaluation (within the past 6 months; panorex as clinically indicated)
5.1.15 Ophthalmology assessment (if clinically indicated)
5.1.16 Psychiatric and Social Work assessment
5.1.17 Refer to Appendix I for disease-specific tests

5.2 Patient Follow Up Evaluation (Post Transplant)

5.2.1 Post-transplant monitoring
a. DHMC BMT SOPs for monitoring and evaluation of allogeneic HSCT recipients will be followed.
b. Re-evaluation: Disease re-evaluation will be performed on days +30, +60, +100, +180, +365 (all visits +/- 15 days) and as otherwise clinically indicated. Disease assessment testing will vary with disease (see appendix IV for appropriate testing).
c. Chimerism assay (VNTR or STR analysis); FISH analysis for y- and x-specific microsatellite markers in the setting of sex mismatched transplants.

5.2.2 Lineage-specific Chimerism Analysis
a. Peripheral blood and/or bone marrow will be obtained on days +30, +60, +100, +180 and +365 (all visits +/- 15 days) (and as otherwise clinically indicated) to determine the degree of ***donor-host chimerism in lymphoid and myeloid lines*** using PCR analysis of microsatellite variable number of tandem repeat (VNTR) or short tandem repeat (STR) markers.
b. Serial lineage-specific post-transplant chimerism analysis will be performed on peripheral blood (preferred) or bone marrow (if WBC inadequate) using PCR of informative minisatellite regions (VNTR or STR) to identify differences between the donor and recipient. Complete donor chimerism (CDC) will be defined as the presence of at least 95% donor DNA in the T-lymphoid (or unfractionated) sample analyzed.

This lineage-specific chimerism analysis, allowing direct characterization of asynchronous lymphoid and myeloid engraftment, will require the specific enrichment of myeloid (CD33+) and T-lymphoid (CD3+) cells prior to performing chimerism testing. Cell enrichment kits (RosetteSep: Stem Cell Technologies, Vancouver, BC), utilizing antibody-mediated cell separation and density centrifugation, will be used to select the cells of interest.

5.2.3 Graft-versus-Host Disease Evaluation

a. Post-transplant acute GvHD assessment (diagnosis and grading) will be undertaken as outlined in the DHMC BMT SOP for the Diagnosis, Grading and Treatment of Acute Graft-versus-Host Disease.
b. Day 100 screening for chronic GvHD (tests below) will be performed at the discretion of the treating physician, based on clinical indication, per DHMC BMT SOP for Chronic GvHD Diagnosis and Treatment. Recommended evaluation for GvHD is listed below:
    1. Skin Biopsy : 4mm punch biopsy- iliac crest/forearm/back
    2. Oral Biopsy from lower lip if clinically indicated, based on symptoms or history of acute GvHD.
    3. Schirmer test if clinically indicated, based on symptoms or history of acute GvHD.
    4. CBC and liver associated enzymes: AST, bilirubin, alk. Phos.
    5. Pulmonary function tests (ABG, if clinically indicated)
    6. Gynecologic evaluation if clinically indicated
    7. Karnofsky score
c. Screening and monitoring beyond day 100 will include the following workup, performed at minimum every 3 months for 2 yrs, then every year x 5 yrs or relapse, whichever occurs first:
    1. Physical exam
    2. CBC/differential, reticulocyte count
    3. Liver, renal and comprehensive metabolic panel
    4. Additional tests and disease staging studies will be performed as clinically indicated by the treating physician.

6.0 STUDY CALENDAR

	Pre-Study[a]	Day +30 (+/- 15)	Day +60 (+/- 15)	Day +100 (+/- 15)	Day +180 (+/- 15)	Day +365 (+/- 15)	Short Term Follow-up[j] (Until Relapse)	Long Term Follow-Up[k] (Yearly for Survival)
Informed consent	X							
HLA typing (molecular)	X							
Pathologic Evaluation[b]	X							
Physical Exam	X	X	X	X	X	X	X	
Disease Specific Staging[c]	X							
Bone Marrow Biopsy	X	X	X	X	X	X		
EKG	X							
ECHO or MUGA	X							
Pulmonary Function Test with DLCO	X							
Dental Evaluation[d]	X							
Mammogram[e]	X							
PAP smear[e]	X							
PSA[f]	X							
Psychiatric and Social Work Assessment	X							
Opthalmology Assesment[e]	X							
CBC w/Diff	X	X	X	X	X	X	X	
PT and PTT	X							
CMP	X	X	X	X	X	X	X	
LDH	X	X	X	X	X	X	X	
B-HCG (serum)[g]	X							
Creatine Clearance from 24 hour urine	X							
Serologic Testing[h]	X							
Antibody Testing[i]	X							
ABO Rh	X							
Reticulocyte Count		X	X	X	X	X		
Chimersim Testing (from peripheral blood or bone marrow)		X	X	X	X	X		
Survival Check								X

a. Based on clinical considerations, each patient will begin treatment on this protocol within a clinically relevant timeframe following completion of the pre-transplant evaluation
b. Pathologic evaluation confirming the diagnosis will be based on institutional diagnostic evaluations per disease
c. Disease staging varies by disease, refer to appendix I
d. Dental evaluation must be within past 6 months; panorex as clinically indicated
e. AS CLINICALLY INDICATED
f. For males 40 years or older with first degree family history of prostate cancer
g. In females of child-bearing potential
h. Serologic Testing includes the following: IgM, IgG, CMV, HSV, VZV, toxoplosmosis
i. Antibody Testing includes the following: Hep A, HBV, HCV, HIV, HTLV I/II, VDRL (or RPR)
j. Short term follow-up will occur every three months for two years following 1 year visit, then every year for 5 years or until death or relapse, whichever occurs first.
k. Long term follow-upwill consist of yearly survival checks

7.0 TREATMENT PLAN

7.1 Patient Treatment Plan

The patient will be admitted to the hospital as clinically indicated during administration of the nonmyeloablative conditioning regimen, stem cell infusion and immediate post-transplant period. A central venous catheter will be placed prior to the start of therapy (triple lumen Hickman catheter preferred).

7.1.1 **Supportive Measures - Infectious Disease**

a. Infectious Disease SOP: DHMC BMT SOP for infection prophylaxis and empiric selective gut decontamination in allogeneic HSCT recipients will be followed.

b. Fungal cultures: Surveillance fungal blood cultures will be obtained at admission in the following high-risk patients:
- Steroid treatment for $\geq$ 3 months duration
- History of prolonged neutropenia (ANC < 500 for > 21 days)
- Neutropenia of uncertain duration

c. VRE: Stools will be tested for VRE if clinically indicated.

d. Transfusion of Blood Products: Leukocyte reduced and irradiated blood products will be used. CMV sero-negative recipients of CMV sero-negative stem cells will receive CMV negative blood products, if available.

7.1.2 **Preparative regimen**

Fludarabine**:** Fludarabine will be administered at 25mg/m^2/day IV on days –6, -5, -4, -3 and -2 as an IV infusion over 30 minutes each day for 5 days. For potential toxicities, see Section 9.0.

Cyclophosphamide: Cyclophosphamide will be administered at 1g/m^2/day IV over 2 hours on days -3 and -2. All patients will receive hydration as outlined below (section 7.3.2.1). Mesna will be given for hemorrhagic cystitis prophylaxis (outlined in section 7.3.2.2). For potential toxicities, see Section 9.0. Recommended supportive anti-emetic therapy with Cyclophosphamide will be as follows:
- Dexamethasone 10mg IV/po 30 min. prior to Cyclophosphamide
- Dolasetron 100mg IV/po 30 min. prior to Cyclophosphamide
- Lorazepam 0.5-2mg IV/po 30 min. prior to Cyclophosphamide, then q4-6h prn for breakthrough nausea or anxiety
- Prochlorperazine 5-10mg po **OR** Promethazine 12.5-25mg IV q6h prn for breakthrough nausea/vomiting

7.1.3 **Transplant**: The allogeneic peripheral blood stem cell product will be infused on Day 0. Stem cell infusion preparation and administration will be performed as outlined in the DHMC SOP for PBSC Reinfusion.

7.2 Treatment Schema

7.2.1 Day –6 through day -2

7.3.1.1 Fludarabine 25 mg/m^2 IV daily over 30 minutes. (If serum creatinine is 1.6-2.5 mg/dl, fludarabine is reduced to 20 mg/m^2 daily x 5 days)

7.2.3 Day –3 and day -2

7.3.1.2 Recommended hydration: One liter of 0.9% NaCl to be infused over 1-2 hours prior to initiation of Cyclophosphamide, followed by 0.9% NaCl infused at 250 ml/hr for 4 hours post-Cyclophosphamide.

7.3.1.3 Mesna at 20% of Cyclophosphamide dose (200 mg/m^2) as an IV bolus infusion 15 minutes prior to Cyclophosphamide. Repeat same intravenous dosage four and eight hours following Cyclophosphamide. If oral, use Mesna tablets at 40% of Cyclophosphamide dose (400mg/m^2) 2 hours and 6 hours after Cyclophosphamide. If vomiting occurs within 2 hours of oral Mesna intake, repeat dosing is necessary.

7.3.1.4 Cyclophosphamide (cytoxan) 1g/m^2 IV over 2 hours.

7.3 GvHD prophylaxis

7.3.1 Cyclosporine (CSA) 3 mg/kg per day, in divided doses, will be administered IV beginning on day – 4. When feasible, oral CSA at 5mg/kg twice daily will be substituted, as tolerated, with dosage adjustments made to maintain serum levels in the 200-400 mg/ml range. CSA levels will be checked on day -1 and every Monday, Wednesday and Friday, thereafter. A taper will be started between days +60-100 as clinically indicated (see below). The precise cyclosporine schedule will depend on chimerism status and on the presence and severity of acute GvHD encountered (see below).

7.3.2 Methotrexate (MTX) will be administered at a dose of 10mg/m^2 intravenously on days +1, +3, and +6.

7.4 Post-transplant Intervention

7.4.1 The **disease status** and **chimeric status** will be assessed in all patients on days +30, +60, +100, +180, +365 (all visits +/- 15 days) for and as otherwise clinically indicated. Post-transplant decisions pertaining to **withdrawal of immunosuppression and donor lymphocyte infusions (DLI)** will be based both on the rapidity and extent of donor cell engraftment and the patient's overall disease status.

- Disease: Analysis of disease status will be undertaken using standard morphologic, cytogenetic and molecular methods, along with flow cytometry, where applicable, to assess marrow/blood for persistent or progressive disease.
- Chimerism: Donor/recipient chimerism status will be interpreted by lineage-specific VNTR or STR PCR analysis of bone marrow, peripheral blood T cells and granulocytes as outlined in Section 5.3.

7.4.2 **Day #30:** No DLI will be undertaken at day +30. Withdrawal of CSA immunosuppression will be done in the following situations:

- Clear disease progression in the absence of significant graft-versus-host disease (> grade II), warranting a rapid taper of cyclosporine therapy (over 2 weeks).

- Donor T-cell chimerism status of <40%, as this predicts for loss of the graft despite DLI [38]. Patients with this level of donor chimerism and no evidence of disease response or significant GvHD will begin a CSA taper at a rate of 25% every 10 days with intended plateau of the taper if day +60 reassessment demonstrates a >95% level of donor T-cell status.
- Patients with 40-94% donor chimerism on day +30 after transplant and stable yet persistent disease with <grade II GvHD will begin a slow CSA taper (10% per week). *(Patients with >95% chimerism status on day +30 and no disease progression will continue to receive CSA until day +60, with reassessment at that time.)*

7.4.3 **Day #60:**

- Patients demonstrating >95% donor chimerism at day +60 with a complete disease response and ≤ grade II GvHD will begin a CSA taper by 5% every week, with the goal to discontinue CSA by 6-7 months post-transplant, unless a change in disease or chimerism status is noted on subsequent evaluation.
- Patients demonstrating 40-94% donor chimerism at day +60 without evidence of progressive disease or significant GvHD will begin a CSA taper by 10% per week with discontinuation at day +130 in an attempt to prevent graft rejection. Absence of a clear trend toward complete donor T-cell chimerism at 2 week follow-up (ie., <95% donor chimerism) and no GvHD progression will prompt consideration for DLI at 1 x 10e7 cells per kg recipient weight, depending on disease and immunosuppressive status.
- Patients demonstrating <40% donor T-cell chimerism status at day +60 without evidence of disease response or significant GvHD will begin a CSA taper of 25% every 10 days. Subsequent improvement in chimerism status by <20% at 1-2 week follow-up (or ongoing total donor chimerism of < 40%) or the development of clear disease progression will prompt administration of DLI of 1 x 10e7 cells/kg.
- Patients demonstrating imminent graft rejection and/or clear disease progression at day +60 in the absence of > grade II GvHD will undergo rapid tapering of CSA over a 2 week period. Failure to demonstrate a clear response at 1-2 week follow-up (and no GvHD progression) will prompt immediate DLI of 1 x 10e7 cells/kg with subsequent dose escalation as indicated (see DLI dose escalation section below).
- Patients who have undergone withdrawal of immunosuppression at the time of their day +30 assessment (above) who demonstrate <20% improvement in donor T-cell chimerism status or persistent/progressive disease at day +60 despite that intervention will be administered DLI of 1 x 10e7 cells/kg.

7.4.4 **Day #60–100**: Patients demonstrating >95% donor chimerism with disease progression between days +60 and +100 (without grade ≥ II GvHD) will have their CSA tapered (either by 25% every 10 days or 100% over a 2 week period, depending on the extent of disease and tempo of progression). If these patients demonstrate ongoing disease progression at 1-2 week follow-up and no evidence of grade ≥ II GvHD, DLI of 1 x 10e7 cells/kg will be administered.

7.4.5 **DLI / Dose Escalation:** All patients not converting to >95% donor chimerism upon follow-up assessment after withdrawal of immunosuppression will be considered for donor lymphocyte infusion. Patients undergoing DLI will be considered for up to 3 escalating doses of donor T-cells at 1 x 10e7, 5 x 10e7, and 1 x 10e8 CD3+ cells/kg recipient weight at 1-6 month intervals, depending on their overall disease state, chimerism status and extent of graft-versus-host disease, based on subsequent monthly or more frequent follow-up.

Mandatory prerequisites for DLI will include the following:

- Minimal persistence of donor T-cell (at least 1-5% donor CD3 cells) chimerism. Supportive stem cell infusion and/or immune suppression may be utilized to supplement DLI, as clinically indicated.
- Discontinuation of all immunosuppressive therapy for at least 2 weeks without significant GvHD flare (ie., not ≥ Grade II)
- Control of significant leukocytosis as indicated (e.g., with hydroxyurea and/or leukapheresis) for patients in hematologic relapse prior to DLI. Such intervention should be discontinued at least one day prior to initiation of DLI. Patients with rapidly progressive malignancies requiring salvage chemotherapy should have this discontinued at least 3 weeks prior to DLI.
- Karnofsky score ≥ 50%.

Consideration for post-DLI dose escalation will be required until one or more of the following is confirmed:

- Attainment of complete donor (>95-100% T-cell) chimerism
- Presence of ≥ grade II acute GvHD or clinical extensive chronic GvHD
- Regression of malignant disease

Patients with chronic myelogenous leukemia (CML) who have been managed with the tyrosine kinase inhibitor imatinib mesylate (Gleevec; STI-571) at the time of referral will discontinue this agent prior to transplant. In the setting of persistent or progressive disease on post-transplant evaluation, this agent may be reinitiated, if clinically indicated, either alone or prior to DLI.

7.5 Use of donor lymphocyte infusions (DLI) following development of aGvHD: Patients developing grade II or greater GvHD after transplant or after DLI will not receive further lymphocyte add-back (DLI) unless GvHD has been successfully treated (i.e. off steroids) or tumor progression is documented.

7.6 Acute GvHD following DLI: See DHMC BMT SOP on the Treatment of Acute GvHD.

7.7 Hospital Discharge

Recommended guidelines for discharge include the following:

1. Afebrile with ANC > 0.5 x 10^9/l and no active, untreated infection.
2. Patient can perform daily activities at home, with caregiver assistance.

3. Patient able to tolerate oral fluids and medications sufficiently to maintain adequate hydration (goal $\geq$ 2 liters po/day).
4. Patient and caregiver's understanding of outpatient care is adequate.

8.0 MANAGEMENT OF POST-TRANSPLANT COMPLICATIONS

8.1 The major complications of allogeneic transplantation include CMV reactivation, veno-occlusive disease (VOD), acute and chronic GvHD, disease progression, graft failure and opportunistic infection (bacterial/fungal). Patients with these complications will be treated according to DHMC Stem Cell Transplant Standard Operating Procedures (SOPs).

8.2 **Treatment of Acute and Chronic GvHD:**
Patients who develop grade >II GvHD will be treated initially with steroids, followed by other immunosuppressive agents, as clinically indicated. Unless the GvHD has been successfully treated, these patients will not receive additional donor lymphocyte infusions. (See DHMC BMT SOP on the Treatment of Acute and Chronic GvHD.)

9.0 POTENTIAL TOXICITIES, HAZARDS AND DISCOMFORTS

9.1 The patient: The mortality from conventional allogeneic BMT may be as high as 40% or greater. Although we anticipate that this protocol is relatively safe, the procedure, nevertheless, carries some risk. The major hazards (GvHD, VOD, infection, graft failure, and disease progression) will be reviewed in detail by the BMT Attending Physician during discussions with all prospective patients and their families. The major discomforts are those of nausea, mucositis, anorexia, diarrhea, fever and malaise. Side effects of the common drugs used in this regimen include:

Cyclophosphamide
Marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility, nausea and vomiting.

Fludarabine
Myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, neurologic toxicity, and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL and other patients treated with fludarabine.

Ganciclovir
Nausea, anorexia, abdominal discomfort, pancytopenia, neutropenia, peripheral neuropathy, renal damage (reversible on drug discontinuation).

Cyclosporine
Renal impairment, hypertension, painful hands and feet, elevated bilirubin, hypertrichosis, nausea, tremor, seizure, hypomagnesemia, thrombotic thrombocytopenic purpura (TTP).

Methotrexate
Mucositis, myelosuppression, renal impairment, hepatic damage, rash, alopecia, fever, nausea, vomiting, diarrhea, abdominal pain, GI bleeding, intestinal perforation.

Antimicrobials (in general)
Allergic reactions, renal impairment, nausea, vomiting, hepatic damage, marrow suppression
Antithymocyte globulin
Allergic reactions, anaphylaxis, serum sickness, rash, fever, chills, myelosuppression

10.0 WITHDRAWAL FROM THE STUDY

10.1 Withdrawal from the transplant procedure

Participants may withdraw from the trial prior to the recipient's admission to the hospital for the transplant. Thereafter, the nature of the procedure does not permit safe withdrawal from the protocol.

10.2 Withdrawal from experimental protocol

10.2.1 The participant has the right at any time not to participate in the trial. All participants who sign an informed consent will be followed, regardless of their clinical status unless the participant withdraws consent for using their health information for research purposes.

10.2.2 Although all patients that sign an informed consent will be followed, patients will *not* be treated according to the trial if the following occurs:

- The recipient fails to achieve allogeneic marrow engraftment (donor chimerism or mixed chimerism)
- Patients with disease relapse after the day +180 restaging who have active grade > II GvHD or who have failed to respond to a previous donor lymphocyte transfusion
- Patients with disease progression associated with a significant decline in performance status, which negates further treatment on the trial (e.g., lymphocyte add-back)

11.0 RESPONSE CRITERIA AND EVALUATION OF RESULTS

11.1 OBJECTIVES

- To evaluate disease response rate as defined in section 12.3.
- To evaluate engraftment by measuring donor-recipient chimerism in lymphoid and myeloid lineages
- To investigate the effect of donor lymphocyte infusion on donor-host chimerism
- To determine the incidence and severity of acute and chronic graft-versus-host disease (GvHD)
- To assess the following:
    - treatment-related mortality
    - time to progression
    - disease-free survival
    - overall survival
    - regimen-related toxicity

12.0 STATISTICS

12.1 Endpoints – The endpoints of this trial include rates of successful engraftment, complete donor chimerism, graft-versus-host disease (acute and chronic), mortality, toxicity, and tumor response rate. The trial will be amended or closed if excessive toxicity or mortality is determined. The specific toxicity stopping criteria are as follows:

- Greater than 33% incidence of severe (Grade III/IV) graft-versus-host disease at day +30 among the first 6 patients enrolled in this study.
- Greater than 33% mortality at day +60 among the first 6 patients enrolled in this study.

The 7^{th} patient will not be enrolled until the results from the first 6 patients are analyzed and it has been determined that there is no excessive toxicity. If at any subsequent time these toxicity criteria are exceeded, accrual to the trial will be suspended and the DSMB will be notified and asked to make a recommendation for continuing, stopping or modifying the trial.

12.1.1 Engraftment will be defined as neutrophil recovery to > 0.5 x 10^9/L for 3 consecutive days and an untransfused platelet count > 20 x 10^9/L.

12.1.2 Chimeric studies will be undertaken on post-transplant days +30, +60, +100, +180 and +365 (all visits +/- 15 days) and as otherwise clinically indicated, according to the method of lineage-specific chimerism analysis described in detail in Section 5.3.2. Complete donor chimerism will be defined as the presence of at least 95% donor DNA in the T-lymphoid (or unfractionated) sample analysis.

12.1.3 Acute and chronic GvHD will be assessed according to the criteria and grading scales of the International Bone Marrow Transplant Registry, as depicted in Appendix III.

12.1.4 Statistical analysis for the primary endpoints – Straightforward descriptive statistical methods will be used to summarize the primary endpoints. Rates of successful engraftment, complete donor chimerism, graft-versus-host disease (acute and chronic), and toxicity will be computed with 95% confidence intervals. Toxicity will be summarized according to type and grade. In addition, rates of any grade 3 or worse toxicity will be computed. Mortality, measured from the time of transplant, will be assessed using the product-limit method, and the survival curve will be graphed.

12.2 One of the endpoints of this study is tumor response rate as defined in section 12.3. It is expected that a response rate between 40% and 60% would represent a successful outcome and would suggest that further studies be conducted to more completely evaluate efficacy of the experimental treatment (i.e., the treatment will be accepted for further study). The target response rate for this trial is 45%, and a response rate of 20% or less would indicate that the experimental treatment should not be recommended for further study (i.e., the treatment would be rejected).

12.3 Statistical analysis for assessment of tumor response rate will utilize an optimal 2-stage design. In the first stage, 10 patients evaluable for response will be accrued. If 3 or more patients experience tumor response (complete or partial) in the first stage, the study will progress to the second stage; otherwise, the study will be

terminated and the treatment will be rejected. In the second stage, 12 additional patients evaluable for response will be accrued, for a total of 22 patients. If 8 or more patients experience tumor response in the full patient sample, then the treatment will be accepted for further study; otherwise, it will be rejected. Tumor response criteria will vary by disease, as outlined in Appendix I. (Note: patients being transplanted with high-risk disease in first or subsequent remission will not be evaluable for response by standard criteria. In this patient subset, determination of treatment efficacy will be based empirically on the absence or development of relapse by morphologic, immunophenotypic, cytogenetic and/or molecular criteria). Tumor response rate will be determined as the ratio of number of complete and partial responders to the total number of patients evaluable for response. The complete response rate will be determined as the ratio of complete responders to the total number of patients evaluable for response. A 95% confidence interval for the response rate will be computed using exact binomial methods.

12.4 Sample size justification – The sample size is based on Simon's optimal 2-stage design (Simon R. Optimal two-stage designs for phase II clinical trials. Controlled Clinical Trials 10:1-10; 1989.). The probability of early stopping is 0.68 and 0.10 for true remission rates of 20% and 45%, respectively. If the true response rate is 20%, the probability of accepting the treatment for further study is 0.05 (statistical significance level). For a true response rate of 45%, this probability is 0.81 (statistical power). With 22 patients, the 95% confidence interval for the response rate will have a half width not exceeding +/- 21%.

13.0 CHIMERISM STUDIES

13.1 VNTR and STR analysis of post-transplant peripheral blood lymphocytes and bone marrow will be assessed in serial fashion to determine the chimeric status of the patient as a function of time. These studies will be repeated after lymphocyte add back to assess the effect of DLI on host chimeric status as well. The relationship between degrees of donor-host chimerism, GvHD and tumor response will be analyzed.

See Section 5.3 (Lineage-specific Chimerism Analysis).

14.0 REGISTRATION GUIDELINES

14.1 This is a non-randomized study.

14.2 To enter eligible patients or discuss a patient's eligibility, please contact Dr. John Hill or any of the BMT physicians or the BMT Clinical Research Associate (Cynara Nayar, MPH. at 603-650-6240. Eligibility will be documented as part of the admit packet. No patients shall be entered on study without consultation with the Principal Investigator and BMT Research team.

15.0 DATA COLLECTION AND DATA AND SAFETY MONITORING

The Safety and Data Monitoring Committee of the Norris Cotton Cancer Center will review this protocol on a quarterly basis. The Dartmouth College IRB will review this protocol on an annual basis. All data will be maintained in a closed data base with each

subject identified by a unique patient number (UPN), with access to patient names only for those involved in clinical care. All patients will provide informed consent prior to study entry. Necessary information will be forwarded to the North American Bone Marrow Transplant Registry (NABMTR).

16.0 ADVERSE DRUG REACTION (ADR) REPORTING

Investigators are required by law to notify the IRB of adverse drug reactions. In addition, the study investigators should be notified. All investigators are required to report secondary malignancies occurring on or following treatment on NCI-sponsored protocols using commercial drugs. Reporting is to be performed in the same manner as reporting Adverse Drug Reactions, including (within ten working days) completion of the FDA Form 1639.

Reporting requirements and procedures depend upon: 1) whether investigational agents are suspected of causing toxicity, 2) whether the possibility of such a toxicity was reported in the protocol, consent form, or manufacturer's literature [Expected Toxicity], 3) the severity or grade of the toxicity.

All grade 4 and 5 toxicities should be reported to the study investigators immediately.

The FDA form 1639 is used for reporting commercial drug toxicities.

All adverse reactions should also be reported to the local Institutional Review Board.

Each patient must be fully informed concerning this study, including pertinent adverse reactions. All institutional or other Federal regulations and guidelines concerning informed consent will be fulfilled.

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Appendix I: Disease Staging and Response Definitions

Staging

1. CML

a. Physical Exam
b. Bone marrow aspiration and biopsy
c. Cytogenetics
d. BCR/ABL gene rearrangement by PCR

2. AML, MDS

a. Physical Exam
b. Bone marrow aspiration and biopsy
c. Cytogenetics
d. Disease-specific translocation by FISH (as indicated)

3. Multiple myeloma

a. Physical Exam
b. Bone marrow aspiration and biopsy
c. M-protein measurement (SPEP or 24 hour urinary light chain excretion light chain disease)
d. SIEP, UIEP
e. Cytogenetics

4. NHL, HD, CLL (only tests that were abnormal initially, unless otherwise indicated)

a. Physical exam
b. CT scans
c. Bone marrow aspiration and biopsy
d. Cytogenetic analysis (as indicated)
e. Disease-specific translocation by PCR (as indicated)

Appendix I (continued):
Response Definitions

1. CML
    a. Complete response (CR): Resolution of bone marrow and blood morphologic abnormalities and absence of Philadelphia chromosome by standard cytogenetic analysis.
    b. Molecular complete remission: In addition to criteria above for complete remission, BCR/ABL rearrangement by RT-PCR is absent.
2. AML/ALL
    a. Complete response: Resolution of abnormal blood and bone marrow morphology with less than 5% blasts and resolution of disease-specific chromosome abnormality (if present) by standard cytogenetic analysis.
    b. Molecular complete remission: In addition to criteria above for a complete response, disease-specific rearrangement is absent by PCR analysis.
3. MDS
    a. Complete response: Resolution of abnormal blood and bone marrow morphology with less than 5% blasts and resolution of disease-specific chromosome abnormality (if present) by standard cytogenetic analysis.
    b. Partial response: Decrease in bone marrow and peripheral blood blast count by > 50%.
    c. Molecular complete remission: In addition to criteria above for a complete response, disease-specific rearrangement is absent by PCR analysis.
4. Multiple myeloma
    a. Complete response: Bone marrow less than 5% plasma cells, polytypic by immunohistology, and absence of M-protein on electrophoresis and immunoelectrophoresis. Resolution of chromosome abnormality (e.g., chromosome 13 deletion), if present, by standard cytogenetic analysis. Absence of skeletal lesion progression by bone survey.
    b. Partial response: Reduction in M-protein by > 50% (and absence of skeletal lesion progression by bone survey).
5. NHL, HD, CLL
    a. Complete response: Resolution of disease by physical findings and normalization of all previously abnormal clinical tests such as bone marrow examination and computed tomographic scans (CT). In addition, resolution of disease-specific chromosome abnormality (if present) by standard cytogenetics.
    b. Partial response: Reduction in disease volume in all measurable sites by ≥ 50%**,** using product of diameters.
    c. Molecular complete remission: In addition to criteria for a complete response, disease-specific rearrangement is absent by PCR analysis.

Appendix II: Prophylaxis and Treatment of Infection

Antibacterial Prophylaxis:

Ciprofloxacin 750 mg PO BID beginning day 0 until neutrophil recovery
Ciprofloxacin 400 mg IV q12h if unable to take ciprofloxacin by mouth

Bactrim DS one tablet PO every Monday, Wednesday, and Friday, from count recovery until 6 months following BMT (longer if ongoing immunosuppressive therapy and/or chronic GvHD).

Antifungal Prophylaxis:

Fluconazole 400 mg PO/IV QD beginning day 0 until engraftment (if day +30 chimerism status suboptimal, continue until day +75). Fluconazole will be discontinued if started on IV amphotericin.

Antiviral Monitoring and Prophylaxis:

1. CMV antigenemia testing will be undertaken between day +10 and day +100 for pre-transplant CMV seropositive patients or patients receiving CMV seropositive stem cells at transplant. If CMV antigenemia is detected, Ganciclovir will be initiated.
2. HSV positive recipient or donor: Acyclovir 400 mg PO TID (or 250 mg/m^2 IV q12h if unable to take PO) from first day of preparative regimen until discharge; at discharge, change to valacyclovir 500 mg PO QD until day +100.
3. HSV negative recipient and donor: no prophylaxis

Immunoglobulin:

Weekly IgG level surveillance starting day +1 until day +100. When levels fall below 400mg/dL, give IVIG 125mg/kg/wk IV. If IgG is still <400 mg/dL on subsequent checks, increase to IVIG 250 mg/kg (or 500 mg/kg, if necessary) to ensure IgG > 400mg/dL prior to day +100 post-transplant. After day +100 post-transplant, IVIG will only be continued on a monthly basis and in the setting of persistent IgG <400mg/dL . This will not be given beyond day +360 post-transplant.

Fever Regimen:

Fever above 38.2 C or sustained fever with two or more readings above 38.0 C. Any temperature with clinical suspicion of bacteremia: see DHMC SOP for Antimicrobial Infections and Protocol.

Appendix III: GvHD Grading Scale

1. **Acute GvHD (see DHMC SOP for the Diagnosis and Treatment of Acute GvHD).**

   a. If patient develops acute GvHD at any time after BMT, acute GvHD is graded as outlined below.
   b. If patient dies before day 30 and has not developed acute GvHD, then the patient is considered not evaluable for GvHD.

Clinical Stage of Acute GvHD according to Organ System

Stage	Skin	Liver	Intestine
+	Maculopapular rash < 25% of body surface	Bilirubin 2-3 mg/dl	>500-1000 ml diarrhea /day or nausea, anorexia or vomiting with biopsy (EGD) confirmation of upper GI GvHD
++	Maculopapular rash 25-50% if body surface	Bilirubin 3-6 mg/dl	>1000-1500 ml diarrhea/day
+++	Maculopapular rash . 50% of body surface area or generalized erythroderma	Bilirubin 6-15 mg/dl	>1500 ml diarrhea/day
++++	Generalized erythroderma with bullous formation and desquamation	Bilirubin > 15mg/dl	>1500 ml diarrhea/day plus severe abdominal pain with or without ileus

Overall Clinical Grading of Severity of Acute GvHD

Grade	Skin	Liver	Gut
I	1-2	0	0
II	0 0 1-3 1-3 1-3 3	0-1 1 0-1 1 0 X	1 0-1 1 0-1 0 X
III	0-3 0-3 0-3	2-3 0-3 4	0-2 2-3 0-3
IV	0-3 4	0-4 0-4	4 0-4

Appendix III (Continued):

2. Chronic Graft-vs.-Host Disease (See DHMC SOP for the Diagnosis and Treatment of Chronic GvHD).

l. If patient develops chronic GvHD at any time after BMT, chronic GvHD is graded as outlined below.

m. If the patient dies before day 100 and has not developed chronic GvHD, then the patient is considered not evaluable for chronic GvHD.

- Limited chronic GvHD

  Either or both

  - Localized skin involvement
  - Hepatic dysfunction due to chronic GvHD

- Extensive chronic GvHD

  Either

  - Generalized skin involvement, or
  - Localized skin involvement and/or hepatic dysfunction

  Plus

  - Liver histology showing chronic aggressive hepatitis, or
  - Involvement of eye (Schirmers test with less than 5mm wetting), or
  - Involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy, or
  - Involvement of any other target organ

3. Pancytopenia

Occurrence of an absolute neutrophil count less than 500/μl or platelet count less than 20,000/μl at any time after DLI, with the pancytopenia not deemed to be due to underlying hematologic disease or chemotherapy.

Appendix IV: Toxicity Scale

See NCI Common Toxicity Criteria (version 2).