ROSWELL PARK CANCER INSTITUTE

STUDY PROTOCOL TITLE: A PHASE II TRIAL OF REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION WITH FLUDARABINE, MELPHALAN AND LOW DOSE TOTAL BODY IRRADIATION

Roswell Park Cancer Institute Study Number: I 177110

Initial Submission Date: 2/15/11

Amendment 1: 1/4/12

Amendment 2: 10/7/14

Approved By
RPCI IRB and UB-CYIRB

OCT 21 2014

Donald Howdy
Institutional Review Board

Principal Investigator: George Chen, MD
Roswell Park Cancer Institute
Elm and Carlton Street
Buffalo, New York 14263
716-845-8614
George.Chen@roswellpark.org

Sponsor: RPCI

Confidentiality Statement

Network Investigator Signature Page

STUDY PROTOCOL TITLE: **A PHASE II TRIAL OF REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION WITH FLUDARABINE, MELPHALAN AND LOW DOSE TOTAL BODY IRRADIATION**

Protocol Approval and Investigator Agreement

I have read and familiarized myself with this protocol and I agree to conduct the study as described according to Good Clinical Practices (GCP) and International Conference on Harmonisation (ICH) guidelines.

Principal Investigator

	2/15/11
Sign	Date
George Chen	
Print	

Address: Elm & Carlton Street, Buffalo, NY 14263

Phone: 716-845-8722

Fax: 716-845-3272

Email: George.Chen@roswellpark.org

STUDY PROTOCOL SYNOPSIS

Title / Phase	**A PHASE II TRIAL OF REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION WITH FLUDARABINE, MELPHALAN AND LOW DOSE TOTAL BODY IRRADIATION**
Roswell Park Cancer Institute Study Number	**I 177110**
Roswell Park Cancer Institute Investigator	George Chen, MD
Sponsor	RPCI
Study Drug(S)	No investigational drug
Objectives	Primary • To determine the transplant related mortality (TRM) of this novel reduced intensity allogeneic stem cell transplantation (RIT) conditioning regimen in a patient population usually not eligible or unable to tolerate a full myeloablative allogeneic transplant. Secondary • To evaluate clinical response, progression free survival (PFS) at one year, engraftment rate, and graft-vs.-host disease (GvHD) incidence with this novel RIT regimen that utilizes a higher dose of melphalan than the previous pilot study across a variety of hematological conditions. • Correlative studies will include chimerism analysis by molecular analysis and evaluation of immune reconstitution by CMV dextramer analysis using flow cytometry.
Study Design	This trial is designed as an open label, non-randomized, single institution study. The primary objective of this study is estimation of the true population TRM rate by day 100 for patients with co-morbidities undergoing RIT who would not be otherwise eligible for or unable to tolerate a full myeloablative transplant. This study will build on our previous experience on Clinical Trial 118807

	which utilized a lower dose of melphalan. Eligible patients will receive conditioning regimen and allogeneic hematopoietic stem cell transplant as the following: Fludarabine 40 mg/m^2/day on days -5 to -2 Melphalan 75 mg/m^2 on day -2 Total body irradiation (TBI) 400 cGy (two fractions of 200 cGy each) on day -1 Hematopoietic stem cell infusion on day 0 Subjects will be followed for at least 12 months after recruitment completion. A total of up to 81 evaluable patients will be accrued. The study design will be a Kepner-Chang Type II Phase II design that proceeds in two stages and stops early only for futility. At the end of the first stage of accrual (41 evaluable patients), data will be reviewed to decide if the study should be terminated due to unacceptable toxicity. Evaluable patients are defined as patients who do not have disease progression within day 100. Non-evaluable patients will be excluded for the primary objective—day 100 TRM evaluation. Non-evaluable patients will be included in the secondary objective analysis including clinical response, PFS at one year, engraftment rate, and GvHD incidence.
Accrual Target / Duration	Accrual target is 81 evaluable patients. The projected accrual is approximately 2-3 patients per month, and therefore recruitment is expected to be complete within 4 years following the study starting point. Subjects will be followed for at least 12 months after recruitment completion, so total study duration will be 5 years.
Evaluation Criteria	Safety Assessments: The primary end point of this study is TRM at day 100. Safety will be evaluated on an ongoing basis with weekly outcome assessment and grading for the first 100 days post transplant. Descriptive statistics will be used to describe safety and other outcomes. Toxicity will be scored according to the Bearman Criteria in Appendix I, oral mucositis by the WHO criteria in Appendix II, acute GvHD according to the criteria in Appendix III and chronic GvHD by the criteria in Appendix IV. Survival: PFS at one year

	Correlative Studies: 1. Myeloid and lymphoid chimerism expressed as a percentage at the following time points (+/- 7 days): day 30, day 100 after stem cell infusion. 2. Immune reconstitution will be evaluated by CMV dextramer analysis using flow cytometry at the following time points (+/- 7 days): baseline, day 30, day 100, and one year after stem cell infusion.
Study Observations	Disease Evaluation: Baseline, day 100 post stem cell infusion and every 3 months afterwards for lymphoma and multiple myeloma patients up to a year. All other patients will have disease evaluation at baseline, day 30, day 100, and one year post stem cell infusion. Serious Adverse Events: From first dose of study drug until 100 days after stem cell infusion. Severe adverse events defined as ≥ grade 3 toxicity by Bearman criteria, ≥ grade 3 acute GvHD, death or readmission due to any cause within day 100 post hematopoietic stem cell infusion will be reported to Clinical Research Services. Hematology: Baseline and daily during hospitalization for transplant. Weekly after discharge until day 100 after hematopoietic stem cell infusion. Important hematologic recovery endpoints: The first of 3 consecutive days of absolute neutrophil count (ANC) $\geq 0.5 \times 10^9$/L; Platelets $\geq 20 \times 10^9$/L after 7 consecutive days with no platelet transfusions; Day of last platelet transfusion. Serum chemistry: Baseline and daily during hospitalization for transplant. Weekly after discharge until day 100 after stem cell infusion. Performance Status: Baseline and daily during hospitalization for transplant. Weekly after discharge until day 100 after stem cell infusion. Physical examination including neurological examination, vital signs, and weight: Baseline and daily during hospitalization for transplant. Weekly after discharge until day 100 after stem cell infusion.
Statistical Considerations	Sample size determination: Since the melphalan dose has been increased to 75 milligrams/meter^2 from previous dose of 50

	milligrams/meter^2, the primary objective is to evaluate day 100 TRM to ensure this regimen remains safe without excessive toxicity. The sample size is determined based on the primary objective: assess the day 100 TRM rate of the study treatment as compared to historical controls. The day 100 TRM for the historical fludarabine/cyclophosphamide (FluCy) is 10% but it has a high day 100 rate of disease progression at 20%. The day 100 TRM for fludarabine/melphalan 140 mg/m^2 (FluMel) is 16% and the day 100 disease progression is 9%. The day 100 TRM from our pilot FluMelTBI trial with 50 mg/m^2 melphalan (protocol No. I-118807) is 11% with a day 100 disease progression rate of 11%. This protocol increases the melphalan dose from 50 mg/m^2 to 75 mg/m^2 with the goal of providing better disease control. We will study whether or not this results a TRM rate between10% and 20%. The sample size calculation is based on testing the hypotheses concerning the proportion of the population who experience a TRM at 100 days: $H_0 : p \geq 20\%,$ $H_a : p < 20\%.$ This two-stage design requires a potential total of 81 patients in order to achieve approximately 80% power to detect differences of 10 percentage points (20% versus 10%). Survival: The estimated distributions of overall and progression free survival will be obtained using the product-limit based Kaplan-Meier method. The Kaplan-Meier methodology will allow for incorporation of the censored observations thereby resulting in a more efficient estimate of the true distribution than if this information was not used. Estimates of quantities such as median duration will be obtained.
Duration of Treatment **Duration of Study**	Duration of treatment: 5 days Duration of study: 5 years

ROSWELL PARK CANCER INSTITUTE

Patient Name: _____

Medical Record No.: _____

Study Protocol Title: A PHASE II TRIAL OF REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION WITH FLUDARABINE, MELPHALAN AND LOW DOSE TOTAL BODY IRRADIATION

			INCLUSION CRITERIA	
			All answers must be "YES or "N/A" for patient enrollment.	**DATE**
Y	**N**	**N/A**	1. The patient must have a diagnosis of one of the following (one must be yes):	
☐	☐	☐	Bone marrow failure disorders: **Acquired bone marrow failure disorders include aplastic anemia, PNH:** **SAA** must have failed at least one cycle of standard immunosuppressive therapy with calcineurin inhibitor plus anti-thymocyte globulin (ATG) if a fully-matched donor is not available.	
☐	☐	☐	**PNH** should not be eligible for a myeloablative HSCT.	
☐	☐	☐	**Hereditary bone marrow failure disorders** include Diamond-Blackfan Anemia, Shwachman-Diamond Syndrome, Kostmann Syndrome, congenital Amegakaryocytic Thrombocytopenia.	
			Other non-malignant hematologic or immunologic disorders that require transplantation:	
☐	☐	☐	**Quantitative or qualitative congenital platelet disorders** (including but not limited to congenital amegakaryocytopenia, absent-radii syndrome, Glanzmann's thrombasthenia)	
☐	☐	☐	**Quantitative or qualitative congenital neutrophil disorders** (including but not limited to chronic granulomatous disease, congenital neutropenia)	

☐	☐	☐	**Congenital primary immunodeficiencies** (including but not limited to Severe Combined Immunodeficiency Syndrome, Wiskott-Aldrich syndrome, CD40 ligand deficiency, T-cell deficiencies)	
☐ ☐	☐ ☐	☐ ☐	Acute Leukemias: **AML** - antecedent myelodysplastic syndrome, secondary AML, high risk cytogenetic abnormalities or normal cytogenetics with high-risk molecular mutations (including but not limiting to Flt3-ITD mutation). **ALL**	
☐ ☐	☐ ☐	☐ ☐	**Chronic Myelocytic Leukemia**: Chronic phase (intolerant or unresponsive to imatinab and/or tyrosine kinase inhibitors) Second chronic phase or accelerated phase who are ineligible for conventional myeloablative transplantation	
☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐ ☐ ☐	☐ ☐ ☐ ☐ ☐ ☐	Myeloproliferative and Myelodysplastic Syndromes: **Myelofibrosis** (with/without splenectomy) with intermediate to high risk features Advanced **Polycythemia Vera** not responding to therapy **MDS** with an IPSS score of INT-2 or higher **MDS** with lower IPSS scores Int-1 or less with severe clinical features; neutropenia, thrombocytopenia, high risk chromosome abnormalities (monosomy 7) **Secondary MDS** with any IPSS score **Chronic myelomonocytic leukemia**	
☐ ☐ ☐ ☐	☐ ☐ ☐ ☐	☐ ☐ ☐ ☐	Lymphoproliferative disease: **CLL, low-grade NHL** (recurrent or persistent) cytotoxic therapy refractory or with less than 6 months duration of CR between courses of conventional therapy. **MM** progressive disease after auto BMT, tandem allo after prior auto **Waldenstrom's** macroglobulinemia (failed one standard regimen). **T or B Cell lymphoma** with poor risk features	

☐ ☐	☐ ☐	☐ ☐	**Hodgkin** Disease: Received and failed frontline therapy Failed or not eligible for autologous transplantation	
☐ ☐ ☐	☐ ☐ ☐	☐ ☐ ☐	2. Histocompatible donor identified: **(Syngeneic donors are not eligible)** Related donor/recipient pairs: must be matched 6 of 6 or 5 of 6 HLA antigens (A, B, DRB1) Unrelated donor/recipient pairs: a single antigen mismatch at HLA A, B, or C, with or without additional allele level mismatch. Must be at least antigen level matched at DRB1. PBSC minimum cell dose is 2 x 10^6 CD34+ cells/kg; marrow 1 x 10^8 nucleated cells/kg	
☐	☐	☐	3. Age ≥ 4 and ≤75 years for blood and bone marrow transplants.	
☐	☐	☐	4. No serious uncontrolled psychiatric illness.	
☐	☐	☐	5. No concomitant active malignancy that would be expected to require chemotherapy within 3 years of transplant (other than non-melanoma skin cancer).	
☐	☐	☐	6. Non-pregnant and non-nursing woman. (Women or men with reproductive potential should agree to use an effective means of birth control).	
☐	☐	☐	7. Patients who have failed a prior autologous or allogeneic transplant are eligible. However, at least 90 days must have elapsed between the start of this reduced intensity conditioning regimen and the last transplant if patient had a prior autologous or myeloablative allogeneic BMT.	
☐	☐	☐	8. At least 2 weeks since prior chemotherapy, radiation treatment and/or surgery.	
☐	☐	☐	9. Informed consent.	

Investigator Signature: _____ **Date:** _____

ROSWELL PARK CANCER INSTITUTE

Patient Name: _____

Medical Record No.: _____

Study Protocol Title: A PHASE II TRIAL OF REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION WITH FLUDARABINE, MELPHALAN AND LOW DOSE TOTAL BODY IRRADIATION

Y	N	N/A	EXCLUSION CRITERIA All answers must be "NO" or "N/A" for patient enrollment	DATE
☐	☐	☐	1. Uncontrolled CNS disease (for hematologic malignancies).	
☐	☐	☐	2. Karnofsky (adult) or Lansky (for ≤16 years) performance status < 50%.	
☐	☐	☐	3. Diffusing Capacity of the Lung for Carbon Monoxide (DLCO) < 40% predicted, corrected for hemoglobin and/or alveolar ventilation.	
☐	☐	☐	4. Cardiac: left ventricular ejection fraction < 40%.	
☐	☐	☐	5. Bilirubin, liver alkaline phosphatase, SGOT or SGPT ≥ 3 x upper limit of normal.	
☐	☐	☐	6. Child's class B and C liver failure (see appendix no. VII).	
☐	☐	☐	7. Calculated creatinine clearance < 40 cc/min by the modified Cockroft-Gault formula for adults or the Schwartz formula for pediatrics (see appendix no. VII).	
☐	☐	☐	8. Patients who have received maximally allowed doses (given in 2 Gy fractionations, or equivalent) of previous radiation therapy to various organs as follows:	

	Adult[1]	Pediatric (≤ 18 yrs)[1]
Mediastinum	40	21
Heart[2]	36	26
Whole lung (s)	12	10
Small bowel[2]	46	40
Kidneys	12	10
Whole liver	20	20
Spinal Cord[1]	36	36
Whole Brain	30	30

Abbreviations and Footnotes: [1] Dose in Grays (Gy); [2]any volume

			Patients who previously have received a higher than allowed dose of radiation to a small lung, liver and brain volume, will be evaluated by the radiation oncologist to determine if the patient is eligible for study.	
☐	☐	☐	9. Uncontrolled diabetes mellitus, cardiovascular disease, active serious infection or other condition which, in the opinion of treating physician, would make this protocol unreasonably hazardous for the patient.	
☐	☐	☐	10. HIV positive.	
☐	☐	☐	11. Patients who in the opinion of the treating physician are unlikely to comply with the restrictions of allogeneic stem cell transplantation based on formal psychosocial screening.	
☐	☐	☐	12. Females of childbearing potential with a positive pregnancy test.	

Study participant meets all entry criteria: ☐ **Yes** ☐ **No**

Investigator Signature: _____ **Date:** _____

Table of Contents

Appendices

GLOSSARY OF ABBREVIATIONS

AML	Acute Myeloid Leukemia
ANC	Absolute Neutrophil Count
ATG	Anti-thymocyte globulin
BMT	Blood or Marrow Transplantation
BU	Busulfan
CLL	Chronic Lymphocytic Leukemia
CMV	Cytomegalovirus
CY	Cyclophosphamide
DLI	Donor Lymphocyte Infusion
DLT	Dose Limiting Toxicity
DVT	Deep Vein Thrombosis
EBV	Epstein Barr Virus
FK506	Tacrolimus
FLU	Fludarabine
G-CSF	Filgrastim
GM-CSF	Sargramostim
GvHD	Graft-versus- Host Disease
GvL	Graft-versus- Leukemia
GvM	Graft-versus- Malignancy
HSCT	Hematopoietic Stem Cell Transplantation
MDS	Myelodysplastic Syndrome
MM	Multiple Myeloma
MMF	Mycophenolate
MTD	Maximum Tolerated Dose
MUD	Matched Unrelated Donor
NHL	Non Hodgkin Lymphoma
NRM	Non Relapse Mortality
PBSC	Peripheral Blood Stem Cell
PMN	Polymorphonuclear Cell
PNH	Paroxysmal Nocturnal Hemoglobinuria
RIT	Reduced Intensity Transplantation
RRT	Regimen Related Toxicity
SOP	Standard Operating Procedure
SOS	Sinusoidal Obstructive Syndrome
TRM	Transplant Related Mortality
VOD	Veno-occlusive Disease

PROTOCOL SUMMARY

Title: **A PHASE II TRIAL OF REDUCED INTENSITY ALLOGENEIC STEM CELL TRANSPLANTATION WITH FLUDARABINE, MELPHALAN AND LOW DOSE TOTAL BODY IRRADIATION**

Precis:

Reduced intensity conditioning during allogeneic hematopietic stem cell transplant (HSCT) reduces transplant related mortality (TRM) by relying more on the graft-versus-malignancy effect rather than the conditioning regimen to eradicate disease. Reduced intensity conditioning regimens can provide a curative option for patients with hematologic malignancies who might otherwise not be candidates for myeloablative (intensive) transplantation because of age or poor performance status. Since the ability to perform transplant safely is the primary measure of efficacy for reduced intensity conditioning, TRM is the appropriate measure of efficacy of the conditioning regimen. Reduced intensity transplantation (RIT) is also the standard of care for certain bone marrow failure states. However, at minimal levels of conditioning intensity such as with the fludarabine/cyclophosphamide (FluCy) or the Fludarabine/200 cGy Total Body Irradiation (Flu/200cGyTBI) regimens, high rates of relapse have been noted, particularly in the myeloid malignancies (AML/MDS). Further improvement in reduced intensity transplantation technique could therefore be achieved by greater cytoreduction (by moderately increasing conditioning intensity) or to hasten the development of GvL (by reducing the time to complete donor lymphoid chimerism). In our initial pilot trial of reduced intensity allogeneic stem cell transplantation (RIT), we explored a lower dose melphalan of 50 mg/m^2 in combination with fludarabine (40 mg/m^2/day x 4 days) and a lower dose TBI (400 cGy) (FluMelTBI) (Protocol No. I-118807). While the regimen was safe (Day 100 TRM=11%), the disease progression rate at day 100 was at 11%. **In this trial, we propose to increase the melphalan dose to 75 mg/m^2 in combination with fludarabine (40 mg/m^2/day x 4 days) and low dose TBI with the intention to improve cytoreduction and hence improve the disease control rate, while preserving the low rate of TRM.** Therefore, TRM will remain the primary objective and day 100 response rate, PFS at one year, engraftment rate, and GvHD incidence are the secondary objectives.

Objectives:

Primary: To determine the TRM of this RIT combination in a patient population who would be at risk for excessive TRM during a full myeloablative allogeneic transplant.

Secondary: To evaluate clinical response, PFS at one year, engraftment rate, and GvHD incidence with our RIT regimen across a variety of hematological conditions. Correlative

studies will include chimerism analysis by molecular analysis and evaluation of immune reconstitution by CMV dextramer analysis using flow cytometry.

Rationale For Regimen Dose Selection:

Fludarabine, a purine nucleoside analog is a potent immunosuppressant that will help prevent donor cell rejection by the recipient. Doses range from 120-200 mg/m^{2}over 4 or 5 days and this protocol will utilize 40 mg/m^2/ per day x 4 days.

Melphalan is an alkylating agent and doses above 140 mg/m^2 are considered myeloablative. In our initial pilot trial of reduced intensity allogeneic stem cell transplantation, we evaluated a lower dose of 50 mg/m^2 in combination with fludarabine (40 mg/m^2/day x 4 days) and lower dose TBI (400 cGy) (Protocol No. I-118807). While the regimen was safe (The day 100 TRM is 11%), the day 100 disease progression rate is 11%. In this trial, we propose to increase the melphalan dose to 75 mg/m^2 x 1 dose in combination with fludarabine (40 mg/m^2/day x 4 days) and lower dose TBI (400 cGy) (2 doses over 1 day) with the intention to improve cytoreduction and improve disease control while preserving the low rate of TRM.

Total Body Irradiation (TBI) is considered myeloablative above 500 to 600 centiGray (cGy). There is extensive experience with lower dose TBI with fludarabine in nonmyelobalative conditioning, usually at 200 cGy. The TBI dose in this study will be 400 cGy administered in 2 doses of 200 cGy. The conditioning intensity, even with 400 cGy TBI, is less than half that of a standard myeloablative Mel 140 mg/m^2/1200 cGy TBI regimen. We therefore consider the dosing range of our regimen to lie within the parameters that define "reduced intensity" and to be a regimen that will not generate excess TRM.

Trial Design:

A total of up to 81 evaluable patients will be accrued. The study design will be a Kepner-Chang Type II Phase II design that proceeds in two stages and stops early only for futility. At the end of the first stage of accrual (41 evaluable patients), data will be reviewed to decide if the study should be terminated due to unacceptable toxicity.

Schedule:

Fludarabine 40 mg/m^2/day on days -5 to -2 (4 total doses)
Melphalan 75 mg/m^2 on day -2 (1 total dose)
TBI 400 cGy (two fractions of 200 cGy each) on day -1
Hematopoietic stem cell infusion on day 0

OUTCOMES

1. TRM in the first 100 days.
2. Engraftment rate.
3. Evaluation of clinical response, one year PFS, GvHD incidence, chimerism analysis, and immune reconstitution.

ELIGIBILITY, SAFETY, EFFICACY, STATISTICS

Please see the relevant sections in the body of the protocol.

---

1. INTRODUCTION

Early allogeneic Hematopoietic Stem Cell Transplant (HSCT), beginning in the late 1960s, utilized a high-dose pretransplant preparative (or conditioning) regimen to deliver myeloablative anti-leukemic treatment. The fully myeloablative cyclophosphamide plus total body irradiation (TBI) regimens pioneered by Thomas, et al resulted in donor cell engraftment but still resulted in small level of relapse.[1] Although increasing the dose of conditioning intensity reduced relapse rates, overall survival was not improved because of higher non relapse mortality or treatment related mortality (TRM).[2] Regimen related toxicities (RRT) limited the application of conventional allogeneic HSCT to patients less than 50-60 years of age, thereby excluding the majority of patients with transplant appropriate diseases. By the 1990s several lines of evidence suggested that allogeneic HSCT had a potentially curative graft-versus-leukemia (GvL) effect in addition to the anti-leukemic action of the myeloablative conditioning regimen. Leukemic relapse was higher in patients who do not develop acute or chronic graft versus host disease,[3-5] who undergo syngeneic (identical twin) transplant[6-8] and who have T-cell depleted allotransplants [3,9] due to lack of a GvL effect. Definitive 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 which generate a GvL effect and eradicate the relapsed disease.[10-15] By the late 1990s, growing confidence in the GvL effect led to the design of several less intense or reduced intensity conditioning regimens designed not to completely eradicate the malignancy but more to provide sufficient immunosuppression to permit donor engraftment, particularly the lymphoid compartment. This strategy reduces immediate RRT with preservation of the GvL effect.[16]

RIT regimens are characterized by: 1) Reversible myelosuppression and autologous hematopoietic recovery without allogeneic stem cell support (usually within 28 days). 2) Mixed chimerism in a proportion of patients early after transplant. 3) Low rates of non-hematologic toxicity.[17] We now have over a decade of data pertaining to reduced intensity conditioning regimens and over 10,000 reduced intensity transplants have been reported worldwide.

A spectrum of RIT regimens exist with varying immunoablative and myeloablative potential. The allogeneic conditioning regimen can be reduced in intensity yet still achieve lymphoid and myeloid engraftment. Initially, up to 17% of patients conditioned with 200 cGy low-dose TBI alone rejected the transplant,[18] but the incidence was decreased to < 3% with the addition of fludarabine (Flu).[19] Rejection rates for Flu–based conditioning regimens are <5% suggesting that Flu (120-200 mg/m^2) can play a critical role in the establishment of engraftment in RIC transplantation. Flu, as the immunosuppressive component of the conditioning, has been successfully combined with variety of other agents (TBI or alkylating agents) to create regimens of different intensities. Of the alkylating agents, cyclophosphamide, busulfan and melphalan have been frequently used. Cyclophosphamide is the most immunosuppressive; whereas busulfan and melphalan provide greater degrees of cytoreduction. FluCy is extremely immunoablative but not very stem cell toxic. Using the standard FluCy regimen, Childs et al showed that donor T-cell engraftment precedes myeloid engraftment and that GvHD and GvL effects only occurred after full donor T-cell chimerism.[20] In contrast, the Seattle group, using low-dose TBI regimens reported the early donor myeloid engraftment, followed later by T-cell engraftment, demonstrating that the regimen was more myelosuppressive than immunosuppressive.[18,21,22]

RIT is generally used when conventional myeloablative conditioning would not be tolerated (due to age or comorbidities) or when conventional myeloablative conditioning is not required (bone marrow failure states, indolent malignancies). **The primary measure of efficacy of RIT is the ability to allow safe transplantation. Consequently, a TRM <20% is conventionally considered an appropriate measure of efficacy of reduced intensity conditioning regimens.**

RIT is dramatically safer in patients who are ineligible for standard myeloablative conditioning regimen because of advanced age or co-morbid illness. The Charlson Comorbidity Index (CCI) has been used to evaluate outcomes in myeloablative and RIT and has proven useful in predicting TRM and overall survival in related and unrelated transplants in a group of patients who are increasingly older and heavily pretreated.[23,24] However the CCI lacks sensitivity; only 35% of RIT patients had co-morbidities identifiable by the CCI and 12% for myeloablative transplants. A recent refinement has been the validated HCT-specific comorbidity index (HCT-CI) which is more discriminant than the CCI at low scores.[25] Stratifying by HCT-CI allows improved prognostication of TRM. Age and disease stage are independent of the HCT-CI. Twenty eight percent of patients were found to have a pre-transplant HCT-CI of 3 or 4 and were found to have a 2-year TRM of 40-43%; in contrast the remaining 72% of patients had a pretransplant HCT-CI of 0 to 2 and were found to have a 2-year TRM between 9-27%.

First generation RIT regimens (FluCy, Flu/ 200cGyTBI and Flu/Bu) have proven to be reliable in donor engraftment, and in reducing TRM. Slow growing malignancies and

nonmalignant conditions generally respond favorably to the first generation RIC regimens. However, we and others have experienced high rates of progression and relapse in the myeloid malignancies (AML/CML/MDS).[26-29] These increased rates in progression and relapse can be attributed either to inadequate cytoreduction (such as with FluCy) or slow achievement of complete donor lymphoid chimerism, a prerequisite for GvL (such as Flu/200cGyTBI, the Seattle regimen). Consequently, further improvement in RIT requires greater cytoreduction (without increasing toxicity) to control the underlying disease until GvL is exerted and/or to hasten the onset of GvL by encouraging the development of rapid donor lymphoid chimerism.[30] Newer generation RIT regimens are being developed by adding myelosuppressive agents such as busulfan or melphalan to Flu/200cGyTBI for greater cytoreduction. The Flu/Bu/TBI conditioning regimen is reported to be safe with 25% day 180 TRM and 50% 1 year overall survival rate. The incidence of relapse at 3 years is still high at 25% with a low 25% 3 year progression free survival (PFS).[31,32] In addition, post-transplant lung toxicity is a concern when combining busulfan and TBI due to the overlapping lung toxicity profile. Melphalan is the alternative myeloablative chemotherapy that can be combined with Flu/TBI to improve cytoreduction. The overlapping toxicities that fludarabine, melphalan, and TBI have are immunosuppression and myelosuppression which are both desired in HSCT. To date, there is no published data using FluMelTBI RIT conditioning regimen.

We are currently completing a pilot RIT trial (I 118807) using the novel combination of fludarabine, low dose melphalan (50 mg/m^2), and low dose TBI (400 cGy). In this novel regimen, we intensified the Flu/200cGyTBI regimen because of high relapse rate and added melphalan and a higher dose of TBI for improved disease control.

Fludarabine, a purine nucleoside analog is an important component of reduced intensity regimens because of its potent immunosuppressive effects at doses ranging from 120-200 mg/m^2 over 4 or 5 days. Melphalan is an alkylating agent. Doses above 140 mg/m^2 are considered myeloablative. **In our initial pilot trial of reduced intensity allogeneic stem cell transplantation, we examined a low dose melphalan of 50 mg/m^2 in combination with fludarabine (40 mg/m^2/day x 4 days) and low dose TBI (FluMelTBI) (Protocol No. I-118807).**

Forty six patients have been accrued on I-118807. The study did not meet the early stopping rules for the 3 planned interim analyses of safety. We compared the day 100 TRM, 1 year TRM, day 100 PFS and 1 year PFS of FluMelTBI to our historical data with other RIT regimens: FluCy and FluMel (Table 1). The day 100 TRM with FluMelTBI is low at 11% compared to our historical data with other RIT regimens (see Table 1 and Figure 1A). The FluCy regimen has a day 100 TRM rate of 10% which is lower than the 16% day 100 TRM rate seen with FluMel (fludarabine, total dose 125 mg/m^2 and melphalan total dose 140 mg/m^2). The FluMelTBI regimen has a lower one year TRM (22%) compared to the FluMel (one year TRM=33%) and FluCy (one year TRM=33%) regimens (Figure 1B). The

median age of patients in I-118807 Flu/Mel/TBI is 10 years older than patients receiving FluMel on RP 98-15 (58 vs. 48 years old), indicating that FluMelTBI is likely better tolerated than FluMel especially in older patients. Although the FluCy day 100 TRM is low, the Day 100 PFS is lower at 66% compared to FluMel (74%) and FluMelTBI (81%), indicating poor disease control with FluCy. The 1-year PFS is similar for the FluMel and FluMelTBI regimens (52 and 49%, respectively), both of which are higher than FluCy (27%) (see Table 1 and Figure 2).

Regimen	Day 100 TRM % (95% CI)	1 year TRM % (95% CI)	Day 100 PFS % (95% CI)	1 year PFS % (95% CI)
FluCy	10 (1-19)	33 (17-49)	66 (51-81)	27 (13-41)
FluMel	16 (11-21)	33 (26-40)	74 (68-80)	52 (45-59)
FluMelTBI	11 (1-21)	22 (8-36)	81 (69-93)	49 (32-66)

Table 1. Comparison of day 100 TRM, 1 year TRM, day 100 PFS and 1 year PFS for patients treated with 3 different reduced intensity regimens: FluCy (RP01-05); FluMel (RP98-15); FluMelTBI (I-118807).

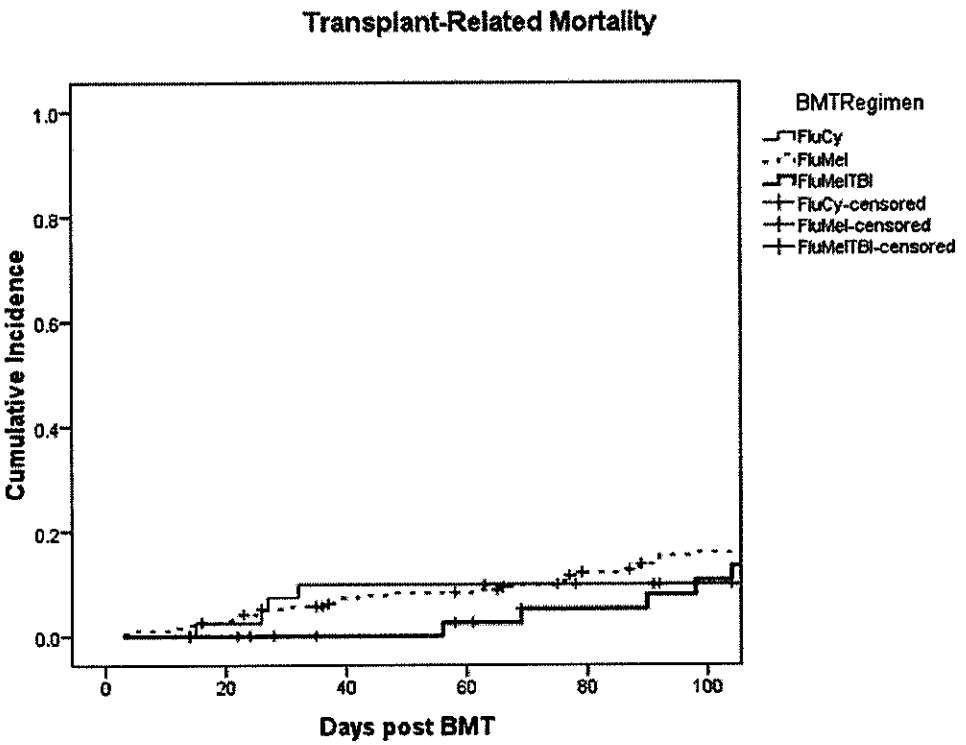

Figure 1A: Cumulative incidence of TRM until day 100 for patients treated with 3 different reduced intensity regimens: FluCy (RP01-05); FluMel (RP98-15); FluMelTBI (I 118807).

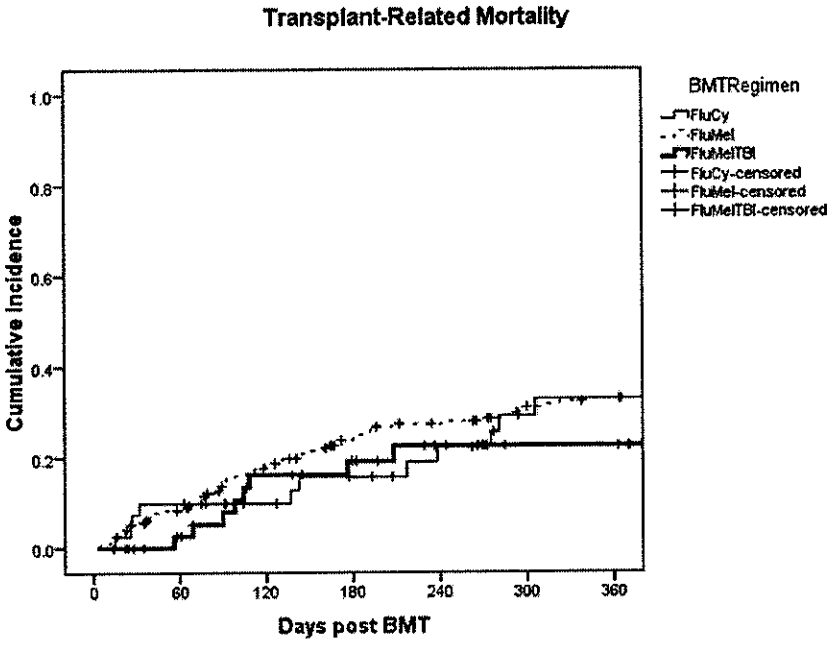

Figure 1B: Cumulative incidence of TRM until 1 year for patients treated with 3 different reduced intensity regimens: FluCy (RP01-05); FluMel (RP98-15); FluMelTBI (I 118807).

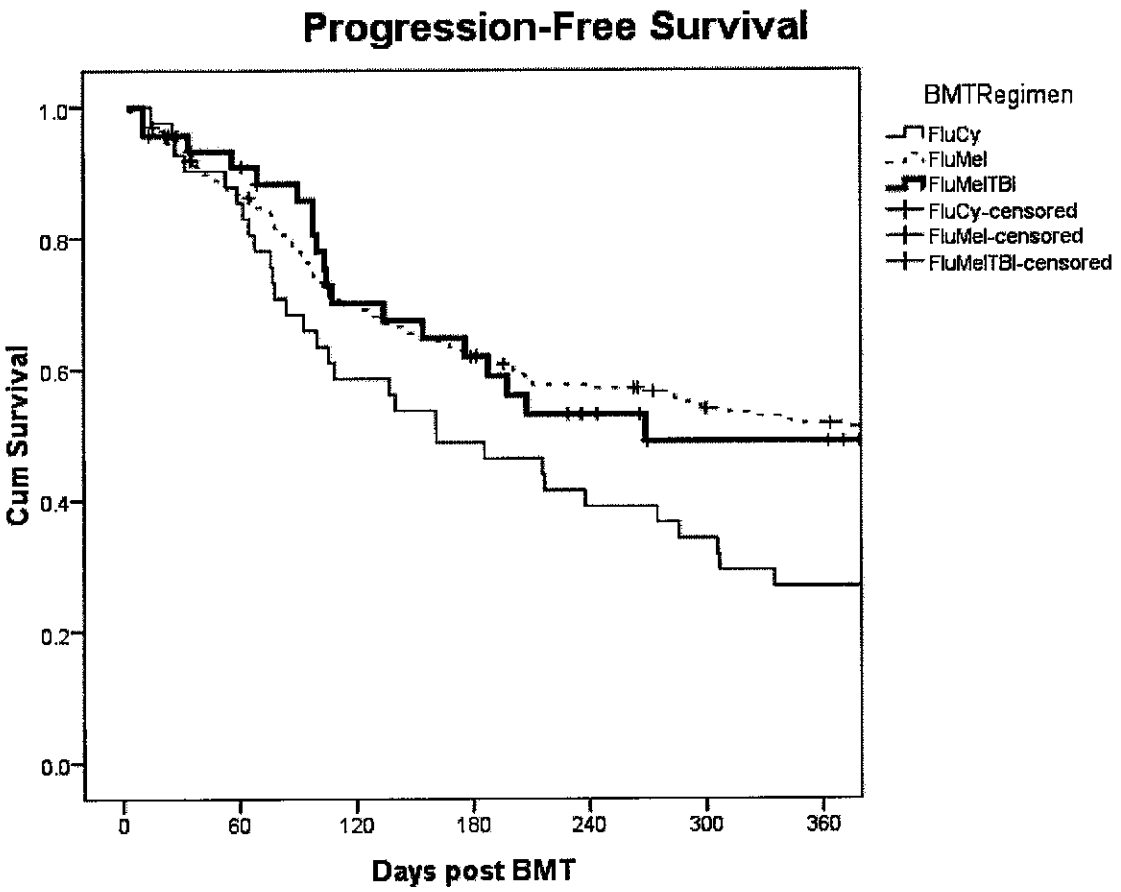

Figure 2: Progression free survival (PFS) at one year for patients treated with 3 different reduced intensity regimens: FluCy (RP01-05); FluMel (RP98-15); FluMelTBI (I 118807).

Based on these results, FluMel/TBI has an acceptable TRM rate, but to improve disease control, this new phase II trial increases the melphalan dose from 50 mg/m^2 to 75 mg/m^2 with the intention to improve the cytoreductive effect and decrease the risk of disease relapse. The role of fludarabine is immunoablative, melphalan is cytoreductive while the TBI is expected to be both immunoablative as well as cytoreductive. Melphalan is dosed in a manner to obtain synergy with fludarabine.[33] This study will build on our previous experience on Clinical Trial 118807 which utilized a lower dose of melphalan.

In the pilot FluMelTBI protocol (I 118807), the percentage of patients who achieved complete donor lymphoid chimerism on day 30 and day 100 was 83% and 90%, respectively (Table 2). We expect to improve the time to development of complete donor lymphoid chimerism with the increased melphalan dose.

Patients	Day 30 Complete Lymphoid Chimerism *(percent)*	Day 30 Complete Myeloid *(percent)*	Day 100 Complete Lymphoid Chimerism *(percent)*	Myeloid Day 100 *(percent)*
37*	31 of 37 ***(84)***	36 of 37 ***(97)***	28 of 31 ***(90)***	29 of 31 ***(94)***

Table 2. Peripheral Blood Lymphoid and Myeloid Chimerism Testing as of May 12 2011. Lymphoid and myeloid chimerism testing was performed on peripheral blood. Peripheral white blood cells were separated by CD3 (T cell, lymphoid) and CD33 (myeloid) isolation. *There are 46 patients on study to date. Seven patients were alive at day 30 and did not have chimerism done. One patient died before day 30, thus 37 patients have day 30 chimerism testing results. The level of sensitivity is 5%. Ninety-five percent and higher is considered complete chimerism. Most patients who did not have chimerism performed had serious medical problems and the chimerism testing was delayed. For Day 100 chimerism testing, 8 patients had died, 5 have not reached day 100 and 2 have not yet been tested but are beyond day 100. A total of 31 patients had day 100 chimerism testing results. We consider this engraftment rate to be excellent for a reduced intensity regimen.

Low dose TBI- TBI will be administered at 400 cGy (two fractions of 200 cGy). TBI doses ranging between 200 and 500 cGy are well tolerated and nonmyeloablative.[34] Fractionation enables tissue recovery and reduces toxicity.

In combination, the dose intensity of the study regimen (measured in terms of the cumulative cytotoxic potential of the Mel and TBI components) is still less than half that of the standard, fully ablative Mel 140/TBI 1200 cGy regimen.[35,36] We consider our study regimen to be safe and consider it a reduced intensity conditioning regimen. Fludarabine, melphalan and TBI are well-characterized agents with a known safety profile and we have experience using this combination at a lower dose of melphalan (50

mg/m^2). Potential overlapping "toxicities" are immunosuppression and myelosuppression, both of which are desirable in an allogeneic transplant. There is a known risk of mild mucositis from the combination of melphalan and TBI, both of which continue to be dosed at half of the standard myeloablative dose. The mucositis in I-118807 has been very low.

2. INDICATIONS FOR RIT IN VARIOUS DISEASE STATES

RIT now has an established role in therapeutic strategies for acute and chronic leukemias, bone marrow failure states (aplastic anemia, paroxysmal nocturnal hemoglobinuria, myelodysplastic syndromes), lymphomas, myeloma, second transplant and immunodeficiencies.

2.1. Bone Marrow Failure States and other non-malignant hematologic or immunologic disorders requiring transplantation

These are preferentially treated with RIT rather than conventional myeloablative HSCT. Primary allogeneic HSCT is appropriate for selected patients with severe aplastic anemia, PNH, and related marrow failure disorders, certain congenital platelet or neutrophil disorders, congenital immunodeficiencies.[37-43,83] Patients with chromosomal breakage syndromes (such as Fanconi Anemia) or Dyskeratosis Congenita can tolerate only very low doses of radiation or chemotherapy due to poor DNA repair capacity. These patients will be excluded from this study.

2.2. AML

Less than 20% of adults ≥ 60 years with AML in first complete remission (CR1) will achieve a 3-year DFS with conventional chemotherapy-based consolidation.[44] While conventional risk factors like cytogenetics and antecedent hematological disorder plays a role, age does appear to be an independent variable.[45-47] Patients over the age of 60 to 65 and those with poor functional status have unacceptable TRM with myeloablative transplant. Reduced intensity conditioning offers an opportunity to achieve a cure in a subset of these patients with outcomes comparable to standard myeloablative allogeneic HSCT.[48,49]

2.3. MDS

MDS is incurable except by alloHSCT. The risk-benefit ratio favors alloHSCT for patients as soon as they progress to more advanced disease (Int-2 or higher IPSS score).[50] It is estimated that only about 5% of MDS patients are eligible to receive myeloablative alloHSCT because of age and other exclusions. RIT may be able to reduce the TRM in this group of patients and allow for wider application.

2.4. MPDs
The only cure for a myeloproliferative disorder is an allogeneic HSCT, which needs to be balanced against the TRM associated with conventional myeloablative HSCT. RIT may be able to reduce the TRM in this group of patients.[51-53]

2.5. NHL
Reduced intensity allogeneic transplantation has a definite role in the treatment of defined subgroups of patients with high-risk NHL with or without a prior autologous transplant.[54] Although aggressive relapse of NHL is not seen frequently as with myeloid malignancies, nevertheless, there is a substantial incidence of relapse in NHL with reduced intensity transplantation.[55,56] This supports efforts to improve on the current Flu/Cy protocol by an increase in cyto-reductive capability.

2.6. Hodgkin disease
Approximately 50% of patients with relapsed and progressive disease after autologous HSCT can be rescued by a RIT.[57]

2.7. Multiple Myeloma
Durable remissions for multiple myeloma can occur after allogeneic HSCT. Previous experience with standard myeloablative allogeneic HSCT was disappointing due to a high early TRM despite achieving durable remissions for survivors.[58,59] Reduced intensity transplantation from suitable related and unrelated donors may permit the development of a curative graft-versus-myeloma effect without high TRM.[60-62] The usual approach is to utilize RIT for selected younger patients with well-matched donors either right after auto transplant ("auto-allo") or after relapse from an auto-transplant. A phase III trial has demonstrated improved overall and disease-free survivals in newly diagnosed myeloma for recipients of a hematopoietic stem-cell autograft followed by a stem-cell allograft from an HLA-identical sibling versus a standard tandem autologous transplant.[63]

2.8. Non-hematologic Malignancy
Patients with solid tumors will be excluded from the current protocol.

2.9. Second Allogeneic Transplants
Second allogeneic transplantation is often offered for relapse after a first allogeneic or autologous transplant and for graft failure. Higher conditioning intensities when used in a second transplant are associated with high rate of RRT and TRM. Therefore, a RIT regimen is an appropriate treatment option for these patients.

2.10. Matched Unrelated Donor (MUD) Transplants
TRM in well matched unrelated donor transplants now approximates that of related transplants because of recent advances in supportive care including the use of fludarabine-based conditioning regimens, tacrolimus-based GvHD prophylaxis, use of

peripheral blood stem cells and high-resolution HLA typing. In one retrospective study of reduced intensity conditioning, overall survival for MUD transplants approximated that of sibling donor transplants.[64]

3. OBJECTIVES:

3.1. Primary objectives:
To determine the TRM of this RIT combination, fludarabine, melphalan and total body irradiation in a patient population usually not eligible for a full myeloablative allogeneic HSCT.

3.2. Secondary objectives:
To evaluate clinical response, PFS at one year, engraftment rate, and GvHD incidence with the proposed RIT regimen across a variety of hematological conditions. Correlative studies will include chimerism analysis by molecular analysis and evaluation of immune reconstitution by CMV dextramer analysis using flow cytometry.

4. PATIENT ELIGIBILITY CRITERIA:

4.1. INCLUSION CRITERIA

1. Diagnosis of a histology documented hematologic malignancy or marrow disorder.

2. Diseases covered:

    a. Bone marrow failure disorders:
        i. Acquired bone marrow failure disorders include aplastic anemia, PNH:
            1. Primary allogeneic HSCT is appropriate for selected patients with severe aplastic anemia.[37,38] However, patients with aplastic anemia must have failed at least one cycle of standard immunosuppressive therapy with calcineurin inhibitor plus anti-thymocyte globulin (ATG) if a fully-matched donor is not available.
            2. Patients with PNH should not be eligible for a myeloablative HSCT.
        ii. Hereditary bone marrow failure disorders include Diamond-Blackfan Anemia, Shwachman-Diamond Syndrome, Kostmann Syndrome, congenital Amegakaryocytic Thrombocytopenia. Fanconi Anemia or related chromosomal breakage syndrome, Dyskeratosis Congenita are excluded from this study due to their poor DNA repair capacity.

1. Fanconi anemia or related chromosomal breakage syndrome: Positive chromosome breakage analysis using diepoxybutane (DEB) or mitomycin C if applicable.
2. Dyskeratosis Congenita: Diagnosis is supported by using either TERC gene mutation in autosomal dominant Dyskeratosis Congenita or X-linked DKC1 gene mutation.

iii. Other non-malignant hematologic or immunologic disorders that require transplantation

1. quantitative or qualitative congenital platelet disorders (including but not limited to congenital amegakaryocytopenia, absent-radii syndrome, Glanzmann's thrombasthenia)
2. quantitative or qualitative congenital neutrophil disorders (including but not limited to chronic granulomatous disease, congenital neutropenia)
3. congenital primary immunodeficiencies (including but not limited to Severe Combined Immunodeficiency Syndrome, Wiskott-Aldrich syndrome, CD40 ligand deficiency, T-cell deficiencies)

b. Acute Leukemias:

i. Resistant or recurrent disease after at least one standard combination chemotherapy regime.[65-70]

OR

First remission patients at high risk of relapse.

i. AML - antecedent myelodysplastic syndrome, secondary AML, high risk cytogenetic abnormalities or normal cytogenetics with high-risk molecular mutations (e.g. Flt3-ITD mutation).[71]

ii. ALL [72,73]

c. Chronic Myeloid Leukemia (CML):

i. Chronic phase (intolerant or unresponsive to imatinib and/or other tyrosine kinase inhibitors), second chronic phase or accelerated phase who are ineligible for conventional myeloablative transplantation.

d. Myeloproliferative and Myelodysplastic Syndromes:

i. Myelofibrosis (with/without splenectomy) with intermediate to high risk features.
ii. Advanced Polycythemia Vera not responding to standard therapy.
iii. MDS with an IPSS score of Int-2 or higher

iv. MDS with lower IPSS scores Int-1 or less with severe clinical features such as severe neutropenia or thrombocytopenia or high risk chromosome abnormalities such as monosomy 7.
v. Secondary MDS with any IPSS scores
vi. Chronic myelomonocytic leukemia.

e. Lymphoproliferative disease:

i. CLL, low-grade NHL (recurrent or persistent) cytotoxic therapy refractory or with less than 6 months duration of CR between courses of conventional therapy.
ii. Multiple myeloma (progressive disease after autologous stem cell transplant, tandem allogeneic transplant after prior autologous stem cell transplant).
iii. Waldenstrom's macroglobulinemia (failed one standard regimen).
iv. T or B Cell lymphoma with poor risk features

54,55,74-76

f. Hodgkin lymphoma:
i. Received and failed front-line therapy
ii. Failed or were not eligible for autologous transplantation

3. Donor eligibility criteria are outlined in section 5. Permissible HLA matching: Related donors- single antigen mismatch at HLA A, B or DRB1; Unrelated donors- a single antigen mismatch at HLA A, B, or C, +/- additional single allele level mismatch at A, B, C or DRB1.

4. Stem cell source criteria are outlined in section 8.1. Minimum goal for PBSC dose is 2×10^6 CD34+ cells/kg of recipient weight; minimum goal for the marrow dose is 1×10^8 nucleated cells/kg of recipient weight.

5. Age ≥ 4 and ≤ 75 years for blood and bone marrow transplants.

6. No serious uncontrolled psychiatric illness.

7. No concomitant active malignancy that would be expected to require chemotherapy within 3 years of transplant (other than non-melanoma skin cancer).

8. Non-pregnant and non-nursing woman. (Women or men with reproductive potential should agree to use an effective means of birth control).

9. Patients who have failed a prior autologous or allogeneic transplant are eligible. However, at least 90 days must have elapsed between the start of this reduced intensity conditioning regimen and the last transplant if patient had a prior autologous or myeloablative allogeneic BMT.

10. At least 2 weeks since prior chemotherapy, radiation treatment and/or surgery.

11. Informed consent.

4.2. EXCLUSION CRITERIA

1. Uncontrolled CNS disease (for hematologic malignancies).

2. Karnofsky (adult) or Lansky (for ≤16 years) performance status < 50%.

3. Diffusing Capacity of the Lung for Carbon Monoxide (DLCO) < 40% predicted, corrected for hemoglobin and/or alveolar ventilation.

4. Cardiac: left ventricular ejection fraction < 40%.

5. Bilirubin, liver alkaline phosphatase, SGOT or SGPT ≥ 3 x upper limit of normal.

6. Child's class B and C liver failure (see appendix no. VII).

7. Calculated creatinine clearance < 40 cc/min by the modified Cockroft-Gault formula for adults or the Schwartz formula for pediatrics (see appendix no. VII).

8. Patients who have received maximally allowed doses (given in 2 Gy fractionations, or equivalent) of previous radiation therapy to various organs as follows:

	Adult[1]	Pediatric (≤ 18 yrs)[1]
Mediastinum	40	21
Heart[2]	36	26
Whole lung (s)	12	10
Small bowel[2]	46	40
Kidneys	12	10
Whole liver	20	20
Spinal Cord[1]	36	36
Whole Brain	30	30

Abbreviations and Footnotes: [1] Dose in Grays (Gy); [2]any volume

Patients who previously have received a higher than allowed dose of radiation to a small lung, liver and brain volume, will be evaluated by the radiation oncologist to determine if the patient is eligible for study.

9. Uncontrolled diabetes mellitus, cardiovascular disease, active serious infection or other condition which, in the opinion of treating physician, would make this protocol unreasonably hazardous for the patient.

10. HIV positive.

11. Patients who in the opinion of the treating physician are unlikely to comply with the restrictions of allogeneic stem cell transplantation based on formal psychosocial screening.

12. Females of childbearing potential with a positive pregnancy test.

5. DONOR ELIGIBILITY CRITERIA/ STEM CELL SOURCE CRITERIA

5.1. Compatibility at the four most informative HLA loci: A, B, C and DRB1 are important for reducing the risk of GVHD and successful transplant outcomes. The A, B, C and DRB1 loci comprise 8 possible alleles (a haplotype being inherited from each parent). One additional locus, HLA-DQ, is also typed to ascertain haplotypes and assist in the search for a compatible donor; however mismatching at DQ has not been shown to be associated with adverse outcomes. High resolution molecular typing (at the allele level) is now the standard of care for unrelated donor searches and allows greater refinement of the search strategy.

5.2. Matched Related Donor: 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 overall survival when compared to full identity at these 3 regions. Related donor/recipient pairs must be matched at 5 of 6 HLA antigens (A, B, DRB1).

5.3. Unrelated Donor: When evaluating patients for unrelated donor transplant, the higher degree of matching, the lower risk of GvHD. The A, B, C, DRB1 and DQB1 loci, comprising 10 possible antigen (with alleles), will be typed for all unrelated transplants. Given the higher risk of TRM in mismatched transplants, RIT is often the best way to mitigate the risk. Data from the National Marrow Donor Program makes it possible to estimate the risk of donor-recipient HLA mismatch at the allele or antigen level.[77] The higher risk from HLA-mismatching must be balanced against the clinical urgency and the patient's risk by the transplant team. At this time, antigen level mismatches at DQB1 do not affect outcomes and will not be used for matching purposes for donor seclection. Thus, the matching required will be at the HLA A, B, C and DRB1 (8 loci). For this

protocol, a single antigen mismatch at HLA A, B, or C, with or without additional single allele level mismatch may participate in this protocol for voluntary unrelated donors (blood or marrow).

5.4. Donor must be healthy and have nonreactive test results for all infectious disease assays as required by state and federal regulations. Donors who screen seropositive 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 to render donation unsafe.

5.6. The donor (or parent if minor) must 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 poor peripheral venous access, may require central venous line placement for stem cell apheresis.

6. TRIAL DESIGN AND STATISTICAL CONSIDERATIONS:

This trial is designed as an open label, non-randomized, single institution study. The primary objective of this study is estimation of the true population TRM rate by day 100 for patients undergoing RIT with co-morbidities or who are otherwise not eligible or unable to receive a myeloablative allogeneic HSCT. In addition to the sample estimate of this probability, an exact 95% confidence interval will also be provided.

6.1. Descriptive analyses

Descriptive statistics such as frequencies and relative frequencies will be computed for all categorical variables. Numeric variables will be summarized using simple descriptive statistics such as the mean, standard deviation and range. A variety of graphical techniques will also be used to display data, ex. histograms, boxplots, scatterplots, etc.

6.2. Accrual

The projected accrual is approximately 2-3 patients per month, and therefore recruitment is expected to be complete by 4 years following the study starting point. Subjects will be followed for at least 12 months after recruitment completion. The total study duration will be 5 years.

6.3. Efficacy analysis

The primary objective of this one-arm Phase II trial is to assess the day 100 TRM rate of the study treatment as compared to historical control. The day 100 TRM for the historical fludarabine/cyclophosphamide (FluCy) is 10% but it has a high day 100 rate of disease progression at 20%. The day 100 TRM for fludarabine/melphalan 140 mg/m^2 (FluMel) is 16% with 9% disease progression rate at day 100. The day 100 TRM from our pilot FluMelTBI trial with 50 mg/m^2 melphalan (protocol No. I-118807) is 11%, however, the day 100 rate of disease progression rate is still somewhat high at 11%. This protocol increases the melphalan dose from 50 mg/m^2 to 75 mg/m^2 to provide better disease control which may result in a higher TRM than 10% but less than 20%. The sample size calculation is based on testing the hypotheses concerning the proportion of the population who experience a TRM at 100 days. Let p represent the proportion of the evaluable population of interest who experience a TRM by day 100 after the beginning of treatment. A true rate of more than 20% is considered unacceptable. A rate of less than 10% is considered promising and evidence of such will deem the treatment worthy of further study. The null and alternative hypotheses corresponding to the design are

$$H_0 : p \geq 20\%,$$
$$H_a : p < 20\%.$$

A total of up to 81 evaluable patients will be accrued. The evaluable population is defined as patients who meet eligibility requirements and are observed for TRM at day 100 after the start of treatment. Patients who have disease progression before day 100 are considered non-evaluable and will be excluded for TRM analysis. Non-evaluable patients will be replaced. The study design will be a Kepner-Chang Type II Phase II (2004) design that proceeds in two stages and stops early only for futility. At the end of the first stage of accrual (41 evaluable patients), data will be reviewed to decide if the study should be terminated due to unacceptable toxicity.

Stage 1: If 8 or more of the first 41 evaluable patients experience a TRM at day 100, it will be concluded that the therapy is not promising and the study will end. Otherwise, the study will progress to the second stage. Depending on the current TRM rate at the time the last patient in Stage 1 is enrolled, study enrollment may be halted until TRM status at 100 days is evaluated in all 41 patients.

Stage 2: We will accrue 40 additional evaluable patients. If 10 or less of the total of 81 evaluable patients experience a TRM at day 100, it will be concluded that p is less than 20% and that the therapy is worthy of further study; otherwise, it will be concluded that there is no evidence to suggest that the therapy is associated with acceptable transplant related toxicity.

With this design, the probability is bounded above by 4.9% of falsely concluding that the true TRM rate is less than 20% and 80.9% is the probability of correctly concluding acceptable toxicity when the true TRM rate is 10%.

6.4. Secondary analyses

The toxicity and overall clinical benefit rates will be computed with corresponding exact 95% confidence intervals based on the methodology of Clopper and Pearson (1934). The estimated distributions of overall and progression free survival will be obtained using the product-limit based Kaplan-Meier method. The Kaplan-Meier methodology will allow for incorporation of the censored observations thereby resulting is a more efficient estimate of the true distribution than if this information was not used. Estimates of quantities such as median duration will be obtained.

6.5. Sample size

The sample size calculation is based on testing the hypotheses concerning the proportion of the population who experience a TRM at 100 days:

$$H_0 : p \geq 20\%,$$

$$H_a : p < 20\%.$$

This two-stage design requires a potential total of 81 patients in order to achieve approximately 80% power to detect differences of 10 percentage points (20% versus 10%).

This protocol will be subject to oversight by the Roswell Park Data Safety Monitoring Board.

7. REGISTRATION AND DATA SUBMISSION

Please contact the Transplant Program Coordinator or Clinical Research Coordinator to enroll patients on the study.

Informed consent: Patient must be aware of the 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 and will have been obtained before this study open.

Histological review: Submission of appropriate tissue samples or outside histo-pathologic slides to confirm the underlying diagnosis. Remission/relapse status should be confirmed within 30 days and no later than 60 days before transplant conditioning starts.

Radiological review: All patients with lymphoma/multiple myeloma must have appropriate radiographic workup prior to registration and must be submitted for review.

Registration procedures: Please confirm eligibility criteria for patients (see Section 4) and donors (see Section 5). Please complete the transplant registration worksheets for patient and donor. When the patient is registered, a patient identification number will be generated. Data pertaining to this protocol will be collected by the BMT research nurses, data coordinators and/ or any other persons assigned by the BMT department head.

7.1 Registration and Data submission

7.1.1 Protocol Date Definitions:

On-Study Date	Start Treatment Date	Off-Treatment Date	Off-Study Date
Date of pre-admission clinic visit after consent signed.	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 or date patient failed to engraft

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

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/ethnicity

7.1.2. Important hematologic recovery endpoints;
First of 3 consecutive days ANC $\geq 0.5 \times 10^9$/L
Platelets $\geq 20 \times 10^9$/L after 7 days with no platelet transfusions
Date of occurrence of severe toxicity (≥grade 3) by Bearman criteria.

7.1.3. Important immunologic recovery endpoints:
Myeloid and lymphoid chimerism expressed as a percentage of donor cells at the following time points (+/- 7 days): day 30, day 100.

Immune reconstitution is evaluated at the following time points (+/- 7 days): baseline, day 30, day 100 and 1 year by the BMT SOC immunophenotyping panel and by analysis of CMV-specific immunity (CMV dextramer).

8. TREATMENT PLAN:

8.1. STEM CELL COLLECTION

Fresh or frozen peripheral blood stem cells (PBSCs) may be utilized. Donors will receive 10 micrograms/kilogram G-CSF daily subcutaneously, starting on transplant day -5. The dose will be rounded to nearest vial size if possible (i.e. 300 micrograms or 480 micrograms). Mobilized peripheral blood stem cells will be collected by apheresis on transplant day "-1" and again on day "0", to achieve a minimum target dose of 2×10^6 CD34+ cells/kilogram of recipient weight. Newer stem cell mobilizing agents (such as plerixafor) may be substituted or added to G-CSF if they are FDA-approved. PBSCs will be unmanipulated with the exception of red cell depletion. CD3+ and CD34+ cell counts as fractions of the total nucleated cells (TNC) will be determined according to established institutional flow cytometry protocols.

PBSCs or Bone Marrow harvest for stem cells are acceptable. The target and minimum doses for bone marrow harvest will be 3×10^8 and 1×10^8 nucleated cells per kilogram of patient body weight respectively. Bone marrow stem cells will be unmanipulated with the exception of red cell depletion. .

Donor lymphocyte collection: donor lymphocytes will not be routinely collected using this protocol. When donor lymphocytes are required for clinical reasons , they will be collected and administered on the RPCI donor lymphocyte protocol.

8.2. PREPARATIVE REGIMEN

8.2.1. 400 cGy TBI based transplant

	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0
Fludarabine (total 160 mg/m^2)	40	40	40	40		
Melphalan (75 mg/m^2)				75		
TBI (400 cGy)					200+200	
Stem cell infusion						X

8.2.2. **Fludarabine** Fludarabine 40 mg/m^2 (actual body weight) is infused over 30 minutes on days -5, -4, -3, and -2 (total dose 160 mg/ m^2).

8.2.3. **Melphalan** Melphalan 75 mg/m^2 (actual body weight) is infused over 30 minutes following fludarabine on day -2.

8.2.4. **Total Body Irradiation (TBI):**

Total Body Irradiation shall be delivered using a nominal photon beam energy of no less than 4 MV. Dynamic or static fields may be used. However, the patient should be entirely included within the dynamic or static treatment field. Lung shields are to be applied in pairs (both AP & PA) with evenly weighted mid-plane dose from each field. When necessary, lung shields can be combined with open (non-shield) fields during the same fraction to achieve the required mid-plane and lung dose. Dose heterogeneity resulting from lung transmission will be assessed using AAPM report number 17 [TG#29], "The physical aspects of total and half-body photon irradiation".

We shall deliver, per fraction of TBI, 200 cGy prescribed to mid-plane at the level of the umbilicus or pelvis, whichever region is thicker. Tissue compensators or other devices shall be used to optimize dose homogeneity within +/- 5 % of the prescribed dose. Dose homogeneity will be assessed at a minimum of six mid plane points at a) head, at largest diameter b) neck, at the level of thyroid notch, c) chest, at the xyphoid process, d) umbilicus or pelvis, whichever is thicker, e) mid thigh, and f) mid-calf. Optional dose points will be located at g) knee, h) ankle, and i) umbilicus or pelvis, whichever is thinner. In addition, a set of lung shields, or an equivalent device, shall be used to ensure that the lungs will receive within 90 to 100 % of the prescribed dose per fraction. The dose rate at the prescription point shall be between 10 cGy and 15 cGy per minute.

Schedule- on day -1. Two fractions of 200 cGy will be given at least six hours apart.

8.2.5. **Hydration regimens** per clinicians' discretion.

8.2. **Supportive medications**: Antiemetics, antimicrobials, etc. will be used according to BMT standard of care.

8.3. **GvHD PROPHYLAXIS**

GvHD prophylaxis will be tacrolimus-based (FK506 or Tacro)[78,79] with micro-dose methotrexate (MTX) and mycophenolate mofetil (MMF) as per the BMT SOP. Cyclosporine may be substituted for tacrolimus in case of intolerance and dosed per BMT SOP.

DAY	-1	0	+1	+2	+3	+4	+5	+6
FK506[1]	X	X	X	X	X	X	X	X
MMF[2]	X	X	X	X	X	X	X	X
MTX[3]			X		X			X

8.3.1. [1]Tacrolimus (FK506): Starting dose will be 0.015 mg/kg (ideal body weight or actual if less than ideal) PO every 12 hours or IV equivalent, from day -1. The tacrolimus dose will be adjusted to target levels between 5-15 ng/ml. In patients who are unable to take oral tacrolimus, the intravenous dose is generally 1/3 of the oral dose every 12 hours. In the absence of GvHD or recurrent disease, tacrolimus will continue until day 60 (for patients at high relapse risk) or until day 100 (low relapse risk) and then taper over 4 to 6 months. Relapse risk is defined in the BMT Program's SOP on GvHD prophylaxis.

8.3.2. [2]Mycophenolate mofetil (MMF)/(Cellcept®)[80]: The dose will be 1000 milligrams orally every 8 hours or 1500 milligrams intravenously every 6 hours for adults. For pediatric patients the dose will be 600 mg/m^2 orally or IV every 8 hours (maximum dose is 1000 mg/dose). Determination of intravenous and oral doses were based on pharmacokinetic levels and calculations to achieve total mycophenolic acid area under the plasma concentration-versus-time curve (AUC) between 30 and 60 microgram/milliliter to prevent graft rejection and graft versus host disease.[81,84] We have measured pharmacokinetic levels on the active drug and the inactive metabolite with Dr. G. Fetterly (unpublished data). Thus, the intravenous dosing is higher based on measured levels. The drug is inactivated by the liver and further dose escalation does not result in higher active drug levels, only higher levels of the inactivated metabolite. The reason for the lower oral dose is gastrointestinal intolerance at doses higher than 1000 mg per mg/m^2 every 8 hours. The MMF starts on day -1 and, in the absence of GvHD or recurrent disease, will continue until day 60 and then stop without taper. *MMF may be withheld at the discretion of the attending physician for patients with cytopenias or other toxicities that are thought to be related to the MMF. The clinical team should attempt to restart this medication when it is thought to be clinically safe.*

8.3.3. [3]Methotrexate (MTX): 2.5 milligrams/m^2 (micro dose) IV on days 1, 3 and 6 will be given for GVHD prophylaxis.

8.4. SUPPORTIVE CARE RECOMMENDATIONS

Prior to initiating therapy, placement of a multi-lumen indwelling silastic catheter is required, preferably a non-tunnelled 5 lumen catheter.

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

8.4.1. Mucosal evaluation and care: Mucositis is expected to be mild to moderate with a RIT regimen. Stomatitis and esophagitis due to herpes virus may be confused with drug-

induced mucositis and viral cultures should be obtained if clinically indicated. Patients should receive acyclovir according to the RPCI BMT standard operating procedure (SOP) for an allogeneic transplant patient.

8.4.2. Antifungal prophylaxis: Antifungal prophylaxis will follow the BMT SOP for allogeneic transplant patients.

8.4.3. Pneumocystis Pneumonia (PCP) Prophylaxis: Anti-PCP prophylaxis should be given according to the RPCI BMT standard operating procedure (SOP) for an allogeneic transplant patient.

8.4.4. CMV Infections: Surveillance and treatment for CMV will follow RPCI BMT standard operating procedure (SOP) for allogeneic transplant patients. Surveillance for CMV by CMV antigenemia or PCR is required once weekly until day +100 and then PRN (as needed). Patients with positive CMV antigenemia should receive induction and maintenance treatment as per the RPCI BMT SOP. A CMV level of 1 or less may be repeated on the next available weekday before initiating therapy and if negative, the patient may be screened again in a week. Treatment dosing will be adjusted for hematological and renal toxicity. CMV surveillance will start no later than day +21 unless patient has not engrafted.

8.4.5. For patients who are pancytopenic prior to transplant, antimicrobial screening prophylaxis should continue through the transplant.

8.4.6. Anti-bacterial propylaxis: Patients will receive anti-bacterial prophylaxis during the transplant period as per the RPCI BMT SOP.

8.4.6. Deep Venous Thrombosis (DVT) prophylaxis will follow the RPCI BMT SOP.

8.4.7. Sinusoidal occlusive syndrome, also known as veno-occlusive disease (VOD) prophylaxis will follow the BMT SOP.

8.5. Allogeneic Stem Cell Reinfusion (Appendix V)

On Day 0 blood or marrow hematopoietic stem cells will be infused. The minimum PBSC dose is 2×10^6 CD34+ cells/kg of recipient weight and the minimum marrow dose is 1×10^8 nucleated cells/ kilogram of recipient weight.

8.6. Chimerism Analysis

Chimerism analysis is clinically useful and important for this study. Samples for chimerism analysis will be obtained on all donor and recipient pairs prior to transplant.

After transplant, serial samples of blood and marrow will be analyzed for chimerism analysis as per the Molecular Diagnostic Laboratory SOP. Lineage specific (separate lymphoid and myeloid) chimerism will be analyzed in blood at the following time points (+/- 7 days): day 30, day 100 and then at unscheduled intervals until full donor chimerism is attained. Unseparated chimerism may also be analyzed in bone marrow (for leukemia and patients with other malignancies if bone marrow was involved previously) whenever a bone marrow is performed. Peripheral blood will be collected in 2 green top tubes for separated chimerism and 1 milliliter of bone marrow aspirate will be collected in 1 purple top tube for non-separated chimerism. Chimerism studies will be performed by the RPCI Moleular Diagnostic lab under Dr. Petr Starostik's direction

Patients not converting to 100% donor T-cell chimerism by day + 180 and/or showing signs of progression of disease after FK506 and MMF withdrawal and/or with refractory viral infection, will be evaluated for DLI. Patients who have active GvHD will not receive DLI.

8.7. Immune Reconstitution

Peripheral blood will be collected at baseline (prior to transplant), day 30, day 100 and one year after transplant for evaluation of immune reconstitution (+/- 7 days). Immune reconstitution will be measured by flow cytometry using markers for T cells (CD3, CD4, CD8, γδ, CD45RA, CD45RO, CD27, HLADr,CD31, CD25, CD127) , B cells (CD19, CD20, CD27, CD38, IgD), and NK cells (CD56, CD16), as well as CMV dextramers. Flow cytometry will be performed by the flow cytometry lab under Dr. Paul Wallace's direction.

9. POTENTIAL TOXICITIES AND THEIR MANAGEMENT

Patients will be managed in the inpatient and outpatient setting. Safety will be monitored on an ongoing basis and discussed, graded and recorded in weekly outcome rounds.

9.1. Management of Graft Versus Host Disease (GvHD):

Approximately 80% of patients will exhibit some manifestation of GvHD. Severe acute GvHD (grade II-III) may occur in up to 20% of patients with an overall mortality of 40-80% 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. Ten percent of patients may experience significant disability due to chronic GvHD that can persist for months to years post-transplant. Standard measures for prevention and treatment of GvHD will be followed.

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 milligrams/kilogram daily for 10-14 days. The goal will be at 1mg per kg at 10 days followed by slow tapering in responders to a maintenance dose of up to 0.25 mg/kg/day, continued for at least 2 weeks from the disappearance of all symptoms of GvHD. FK506/MMF will be maintained or increased during active aGvHD. Refractory GvHD will be treated as per attending physician's discretion and/or BMT SOP.

9.2. Regimen Related Toxicity (Bearman Criteria; WHO mucositis scale; APPENDICES I and II).

9.2.1. Cardiac:
Patients with pre-existing cardiac disease are at significant risk for developing congestive heart failure and arrhythmias. Studies to be obtained when clinically indicated include EKG (to compare voltage to pretreatment EKG), radionuclide ventriculogram, and/or echocardiography.

9.2.2. Renal:
Many of the agents used in this study can cause renal toxicity, which can be minimized by vigorous hydration, avoidance of exposure of multiple nephrotoxic drugs where clinically possible, appropriate drug monitoring and dose adjustment. Kidney damage is usually reversible, but severe cases may require dialysis.

9.2.3. Pulmonary:
"Engraftment syndrome" consisting of hypoxia, pulmonary effusions and/or infiltrates may be seen at the time of donor cell engraftment and should be treated promptly with methylprednisolone 2 milligrams/kilogram daily followed by rapid taper.

Based on our previous protocol, the risk of fatal idiopathic interstitial pneumonitis is estimated to be <10%. Patients with hypoxia or hypoxia with ambulation should be evaluated as soon as possible by CT scan. Whenever possible, histologic confirmation of the diagnosis should be attempted by bronchoalveolar lavage, transbronchial or open-lung biopsy. This would help to exclude infectious causes (cytomegalovirus/fungal) or confirm the diagnosis of hemorrhage.

9.2.4. Hepatic:
Moderate toxicity can occur. More severe, possibly fatal, liver toxicity rarely occur, usually in the form of sinusoidal obstructive syndrome (SOS), also known as veno-occlusive disease (VOD). With these study regimens the incidence of fatal liver toxicity should be well less than 10%. Management of liver toxicity is with continued

ursodeoxycholic acid and standard supportive and symptomatic measures. The clinical team should avoid exposure of patients to other hepatotoxic agents.

9.2.5. CNS:
Fludarabine can cause weakness, paraesthesias 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.

9.2.6. Mucositis:
Many patients will experience mild to moderate stomatitis and dysphagia. Adequate pain relief often requires parental narcotics. Prevention requires aggressive oral hygiene as per RPCI BMT SOPs.

9.2.7. Nausea/Vomiting:
Many patients will experience moderate to severe nausea and vomiting. All patients should receive vigorous antiemetic treatment as per RPCI BMT SOP. At or after neutrophil engraftment, the development of nausea and vomiting must prompt evaluation for GvHD.

9.2.8. Diarrhea:
Most patients will experience moderate to severe diarrhea in the first three weeks, which responds to standard symptomatic therapy. Appropriate studies are needed to exclude infectious causes of diarrhea, especially *C. difficile*. At or after neutrophil engraftment, diarrhea must prompt evaluation for GvHD.

9.3. Other Toxicities:

Infections:
Viral infections (CMV, EBV, respiratory viruses including RSV, influenza virus and parainfluenza virus, BK virus, adenovirus), fungal infections (candida and mould) and bacterial infections are commonly expected in the setting of an immunocompromised host, chemotherapy administration and delayed immune reconstitution. These will be managed according to the RPCI BMT SOP.

Hematologic:
Major ABO incompatibility (recipient vs. donor RBCs) or minor ABO incompatibility (donor vs. recipient RBCs) or both may result in severe hemolysis. ABO incompatible transplants will be identified in advance and will be treated according to the RPCI BMT SOP.

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

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.

Hair:
Some patients, particularly those receiving melphalan and total body irradiation will experience total (reversible) alopecia.

Secondary malignancy:
The cumulative exposure to chemotherapeutic agents and radiation increases the risk of developing leukemia or a second cancer. The incidence of leukemia is probably approximately 5%.

Failure to engraft:
Autologous count recovery is the most likely outcome because this combination is sub-myeloablative.

Toxicities of G-CSF (donor):
These toxicities may include bone pain, headache and myalgias. These can be treated with non-narcotic and narcotic analgesics. Splenic rupture in donors with an enlarged spleen and precipitation of an acute hemolytic episode in donors with sickle cell trait are rare and need to be evaluated.

Toxicities of Apheresis (donor)
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 (sodium citrate), 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. A reaction (such as a rash) to a drug is possible if the patient has an unknown allergy. Blood loss (less than a pint) may rarely occur because of equipment problems which make it impossible to return the blood to the donor. Air entering the blood stream causing air to go to body organs (very rare risk because of safety measures used).

Toxicities of bone marrow harvest (donor)
In the event the patient cannot undergo apheresis or had a poor hematopoietic stem cell collection, a bone marrow harvest will be performed. The risks of the bone marrow harvest include anesthesia (general or spinal), pain, infection and bleeding at bone marrow harvest sites (posterior iliac crests bilaterally).

10. DRUG FORMULATION, AVAILABILITY AND PREPARATION:

10.1. Fludarabine Monophosphate (Fludara®);

A purine nucleoside analog.

10.1.1 Availability:
Fludarabine monophosphate is commercially available as a clear, sterile solution. Each 2 milliliter vial contains 50 milligrams of fludarabine phosphate, 50 milligrams of mannitol, water for injection, and sodium hydroxide to adjust pH to 6.8. Store at 2 to 8°C (36 to 46°F).

10.1.2. Storage & Stability:
Fludarbine phosphate 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.

10.1.3 Preparation:
Fludarbine phosphate vials containing 50 milligrams of fludarabine phosphate, 50 milligrams of mannitol, water for injection and sodium hydroxide to adjust the pH to 6.8. The product may be further diluted for intravenous administration in 100 milliliters or 125 milliliters of 5% Dextrose for Injection USP or 0.9% Sodium Chloride, USP.

10.1.4. 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.

10.1.5. 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 25-40 milligrams 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).

10.2. Melphalan (Alkeran®):

An alkylating agent.

10.2.1 Availability:
Melphalan is commercially available as a powder for injection in 50 milligrams vials.

10.2.2. Storage & Stability:
Intact vials should be stored between 20 to 25°C (68 to 77°F) and protected from light. Following reconstitution with sterile diluent, melphalan hydrochloride solution containing 5 milligrams of melphalan per milliliters is stable for up to 60 minutes at room temperature; this reconstituted solution should not be refrigerated since a precipitate may form at 5°C.

10.2.3. Preparation:
Reconstitute by adding 10 milliliters of diluent provided by the manufacturer to a vial labeled as containing 50 milligrams of melphalan to provide a solution with a concentration of 5 milligrams/milliliters. The diluent should be added rapidly and the vial should be shaken vigorously until a clear solution is obtained. Reconstituted solutions of melphalan should be inspected visually for particulate matter and discoloration prior to administration whenever solution and container permit. Administration should occur within one hour of drug dissolution.

10.2.4. Administration:
IV administration over 30 minutes. Administration should occur within 60 minutes of dissolution.

10.2.5. Toxicity:
Leukopenia. thrombocytopenia. irreversible bone marrow failure, vasculitis, secondary malignancies, vesiculation of the skin, alopecia, pruritis, rash, SIADH, sterility, amenorrhea, nausea, vomiting, stomatitis, diarrhea, hemorrhagic cystitis, anemia, hemolytic anemia, pulmonary fibrosis, interstitial pneumonitis.

10.3. Tacrolimus (Prograf®);

Macrolide compound with potent immunosuppressant properties.

10.3.1. 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 1 milligram/milliliter was stable for 56 days when it was stored at 24-26°C in glass or plastic amber prescription bottles.

10.3.2. Availability:
Tacrolimus is available for oral administration as capsules, containing the equivalent of 0.5, 1, or 5 milligrams of anhydrous tacrolimus and compounded oral suspension containing the equivalent of 1 milligram/milliliter of anhydrous tacrolimus. For IV use, tacrolimus is available as a sterile solution in 1 milliliter ampules containing the equivalent of 5 milligrams 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 15 nanograms/milliliter. 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.

10.3.3. Preparation: For IV use:
Tacrolimus concentrate for injection must be diluted prior to IV infusion. For IV infusion, the concentrate is diluted with 0.9% sodium chloride or 5% dextrose injection to a concentration of 4-20 micrograms/milliliter. 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 matter and discoloration prior to administration whenever solution and container permit.

For Oral use:

Tacrolimus 1 milligram/milliliter suspension is compounded using twenty-four 5 milligram capsules, 60 milliliters of simple syrup, and 60 milliliters of Oraplus® suspension. Capsules are emptied into an 8 ounce amber bottle and dissolved in 8

milliliters of sterile water. Simple syrup and Oraplus® are added and shaken vigorously. Storage is at room temperature for 56 days.[82]

10.3.4. Administration:

Oral therapy should be started as soon as possible after transplantation at a dose of 0.015 milligrams/kilogram (ideal body weight or actual if less than ideal) every 12 hours. In patients unable to tolerate oral therapy, the recommended intravenous dose is one-third of the oral dose administered every 12 hours.

10.3.5. 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 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.

10.4. Mycophenolate (CellCept®, MMF):

A 2-morpholinoethyl ester of mycophenolic acid (MPA), inosine monophosphate dehydrogenase (IMPDH) inhibitor.

10.4.1. Storage & Stability

The MMF powder for reconstitution and reconstituted solution should be stored at 25 degrees Celsius (C) (77 degrees Fahrenheit (F)); however, temperature excursions between 15 to 30 degrees C (59 to 86 degrees F) are permitted (Prod Info CellCept(R), 2003e).

10.4.2. Availability and administration:

Mycophenolate is available for oral administration as capsules containing 250 milligrams of mycophenolate mofetil, tablets containing 500 milligrams of mycophenolate mofetil, and as a powder for oral suspension, which when constituted contains 200 milligrams/milliliter mycophenolate mofetil. Each vial of mycophenolate intravenous contains the equivalent of 500 milligrams mycophenolate mofetil as the hydrochloride salt. Reconstitution and dilution with 5% Dextrose Injection USP yields a solution of mycophenolate mofetil, 6 milligrams/milliliter.

10.4.3. 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.

10.4.4. 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.

10.4.5. 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.

10.4.6. 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.

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

10.4.8. Toxicities:
The principal adverse reactions associated with the administration of mycophenolate 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 mycophenolate, 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 mycophenolate (2000 to 3000 milligrams daily) 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 mycophenolate has been shown to have teratogenic effects in animals, it may cause fetal harm when administered to a pregnant woman. Therefore, mycophenolate should not be used in pregnant women unless the potential benefit justifies the potential risk to the fetus. It is recommended that mycophenolate therapy should not be initiated by the physician until a report of a negative pregnancy test has been obtained.

In patients receiving mycophenolate (2000 or 3000 milligrams) 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) $<0.5 \times 10^3$ / microliter] developed in up to 2.0% of renal, up to 2.8% of cardiac, and up to 3.6% of hepatic transplant patients receiving mycophenolate 3000 milligrams daily respectively. If neutropenia develops (ANC $<1.3 \times 10^3$ / microliter), dosing with mycophenolate 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 mycophenolate 3000 milligrams daily. Gastrointestinal perforations have rarely been observed.

10.5. METHOTREXATE (MTX):

Antimetabolite that interferes with DNA synthesis, repair and cellular replication

10.5.1. Availability and Stability:

Methotrexate LPF® Sodium (methotrexate sodium injection), Isotonic Liquid, Preservative Free, for single use only, is available in 25 milligrams/milliliter, 2, 4, 8, and 10 milliliter vials, containing 50, 100, 200, and 250 milligrams 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 available in 20, 50, and 1000 milligrams 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 milligrams/milliliter, 50, and 250 milligrams 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.

10.5.2. Dosage and Administration:

There is a wide range of dosing of methotrexate up to 10,000 mg per M^2 over 15 to 30 minutes that would include leucovorin rescue. For this protocol, the dose of methotrexate is very low and will not require a leucovorin rescue.

10.5.3. Toxicities:

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

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.

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 alkalinization are essential for safe administration.

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 stomatitis or other severe GI adverse reactions occur.

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.

Skin: erythematous rashes, pruritus, urticaria, photosensitivity, pigmentary changes, alopecia, ecchymosis, telangiectasia, acne, furunculosis.

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.

Other: opportunistic infection, arthralgia/myalgia, loss of libido/impotence, diabetes, osteoporosis and sudden death. A few cases of anaphylactoid reactions have been reported.

11. DATA AND SAFETY MONITORING PLAN

The Principal Investigator (PI) will be responsible for continuous monitoring of the safety of the study.

Patient Outcomes Rounds are held weekly on the transplant unit, at which time all BMT patient care is reviewed, including:

Medications (chemotherapy for conditioning regimens; prophylactic, empiric and therapeutic antimicrobials; graft-versus-host disease prophylactic and therapeutic medications; and possible drug interactions).

Adverse events and/or adverse reactions to any medication, procedure, or other treatment; reports are filed according to RPCI policy and procedure.

Regimen-related toxicity, based on Bearman toxicity grading, and/or Common Toxicity Criteria (CTC) if the toxicity does not correlate with a Bearman grade.

Additional testing or therapies such as biopsies, scans or xrays that will direct therapeutic interventions.

Consent, a properly signed and dated transplant consent.

Patient Psychosocial Status, compliance issues that could compromise patient safety. A pretransplant, a conference is held by the BMT for all BMT patients for the purpose of describing the need for allogeneic patients to obtain lodging within a 30 mile radius of the hospital and to have a caregiver present at all times while the patient is an outpatient up to 100 days following transplant. In addition, psychosocial evaluations are completed on all transplant patients by one of the BMT social worker prior to transplant, to identify any compliance issues.

Safety Monitoring, mandated by the BMT Standards of Care and common clinical practice. These include daily physical examinations, clinical laboratory testing, routine surveillance cultures, therapeutic drug level monitoring (i.e., Vancomycin, Tacrolimus, Tobramycin, Cyclosporine, Sirolimus, Mycophenolate). They are found in the BMT SOP. Patients who have been discharged from the hospital are monitored in the BMT Clinic until all transplant-related issues are resolved and they are returned to the care of their referring physicians.

Performance Status, assignment of KPS/Lansky Score (Appendix VI)

Quality Assurance, the BMT Quality Assurance plan requires quarterly reporting to the BMT Quality Assurance Committee, which in turn reports to the hospital Quality Assurance Committee. Indicators for BMT patient safety monitoring include:

- Patient complaints
- Reportable adverse events (serious)
- Bearman toxicity grade ≥ 3
- Variances in the delivery of standard care that do or do not result in a change in practice
- Readmissions prior to day +100 post transplant
- Deaths occurring prior to day +100 post transplant

- Engraftment of neutrophils and plateletes

Long Term Follow-up is conducted on all transplant patients even after they have returned to the care of the referring physicians. An Annual Transplant Clinic has been established, which provides care for allogeneic patients with chronic complications, as well as assessments to identify clinical problems such as dental, bone, and psychosocial complications.

BMT Patient Outcomes are reported to the Center for International Blood and Marrow Transplant Research (CIBMTR), and/or the National Marrow Donor Program (NMDP). Registry reports are reviewed internally prior to submission to the respective registry. These data are also entered into the RPCI BMT Database, from which patient outcomes are assessed and reviewed on a regular basis. Regimen-related toxicities reported in this fashion have resulted in a number of changes to transplant protocols since 1997, thus decreasing toxicity and improving outcomes in a number of patient groups. Registry reports also establish the efficacy of treatment as measured by overall best response to transplant at day +100 and on subsequent annual reports. The patients' medical records serve as original source documents for all reporting. Audits are conducted every two to three years by the CIBMTR and the NMDP.

12. 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 protocol treatment and placed on follow-up. In this event the reason for withdrawal will be documented, the PI will be notified and the patient will be followed for toxicity, survival, progression and relapse. Any patient with disease progression will be removed from the protocol treatment and placed on follow-up. Document details, including last staging on flow sheets and follow the patient for survival and secondary malignancies.

12.2. 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 or higher toxicity.
Grade 3-4 acute GVHD will be reported by written notification.
Failure to engraft (defined as a patient alive on day 30 with no ANC recovery)

Late graft failure (defined as a patient who had initial ANC recovery but subsequently had a decline in ANC that required additional stem cell support.
Readmission due to any reason up to 100 days post BMT

12.3. Unexpected Toxicity: All severe AEs not listed in expected toxicity will be reported within 72 hours.

13. CRITERIA FOR STUDY EVALUATION

13.1. Disease Response Criteria:
Patients will be followed according to response criteria as referenced in BMT SOP "Standards of Therapy" last updated 2008.
http://internal.roswellpark.org/files/1_2_1/Internal/patient_care/bmt/BMT%20Standards/8-2006%20BMT%20Standards.pdf

13.2. Study Endpoints:
The primary endpoint is Transplant Related Mortality in the first 100 days from day 0 of the transplant. TRM is defined as death due to any cause except for disease progression.

13.3. Safety And Toxicity:
Safety will be evaluated on an ongoing basis with weekly outcome assessment and grading for the first 100 days post transplant. Descriptive statistics will be used to describe safety and other outcomes. Toxicity will be scored according to the Bearman Criteria in Appendix I, oral mucositis by the WHO criteria in Appendix II, acute GvHD according to the criteria in Appendix III and chronic GvHD by the criteria in Appendix IV.

13.4. Engraftment:
Neutrophil engraftment is defined as the first day in which ANC is > $0.5x10^9$/ liter for three consecutive days. Platelet engraftment is the first day that platelets are >20 $x10^9$/ liter for seven consecutive days without transfusion support.
Engraftment is a dynamic process in RIT, and chimerism analysis will be performed at regular intervals. Lineage specific (separate lymphoid and myeloid) chimerism will be analyzed in blood at the following time points (+/- 7 days): day 30, day 100 and then at unscheduled intervals till full donor chimerism is attained.
Primary engraftment failure is defined as lack of evidence of neutrophil or platelet engraftment at day +35, confirmed by a bone marrow biopsy showing <5% cellularity in the absence of persistent malignancy. Late graft failure will be defined as initial neutrophil engraftment by day +35 documented to be of donor origin followed by a drop

in ANC to <500 for more than three days, independent of myelosuppressive drugs, severe GvHD or infection.
Graft rejection is defined as graft failure with documentation of return of recipient hematopoiesis as determined by cytogenetic/chimerism analysis.

14. GENDER AND MINORITY DECLARATION

This protocol does not restrict enrollment on the basis of gender or race.

15. CONFIDENTIALITY

All information provided to the Investigator by RPCI including preclinical data, protocols, CRFs, and verbal and written information, will be kept strictly confidential and confined to the clinical personnel involved in conducting this study, and no disclosure shall be made except in accordance with any right of publication granted to the Investigator. This information may be related in confidence to the IRB/ERC or other committee functioning in a similar capacity. No report or information about the study will be provided to anyone not involved in the study without consent of RPCI except if required by law.

REFERENCES

1. Thomas ED, Buckner CD, Banaji M, et al. One hundred patients with acute leukemia treated by chemotherapy, total body irradiation, and allogeneic marrow transplantation. Blood. 1977;49:511-533.
2. Clift RA, Buckner CD, Appelbaum FR, et al. Allogeneic marrow transplantation in patients with acute myeloid leukemia in first remission: a randomized trial of two irradiation regimens. Blood. 1990;76:1867-1871.
3. Horowitz MM, Gale RP, Sondel PM, et al. Graft-versus-leukemia reactions after bone marrow transplantation. Blood. 1990;75:555-562.
4. Sullivan KM, Storb R, Buckner CD, et al. Graft-versus-host disease as adoptive immunotherapy in patients with advanced hematologic neoplasms. N Engl J Med. 1989;320:828-834.
5. Weiden PL, Sullivan KM, Flournoy N, Storb R, Thomas ED. Antileukemic effect of chronic graft-versus-host disease: contribution to improved survival after allogeneic marrow transplantation. N Engl J Med. 1981;304:1529-1533.
6. Fefer A, Cheever MA, Greenberg PD. Identical-twin (syngeneic) marrow transplantation for hematologic cancers. J Natl Cancer Inst. 1986;76:1269-1273.
7. Gale RP, Champlin RE. How does bone-marrow transplantation cure leukaemia? Lancet. 1984;2:28-30.
8. Gale RP, Horowitz MM, Ash RC, et al. Identical-twin bone marrow transplants for leukemia. Ann Intern Med. 1994;120:646-652.
9. Antin JH. Graft-versus-leukemia: no longer an epiphenomenon. Blood. 1993;82:2273-2277.
10. Cullis JO, Jiang YZ, Schwarer AP, Hughes TP, Barrett AJ, Goldman JM. Donor leukocyte infusions for chronic myeloid leukemia in relapse after allogeneic bone marrow transplantation. Blood. 1992;79:1379-1381.
11. Drobyski WR, Keever CA, Roth MS, et al. Salvage immunotherapy using donor leukocyte infusions as treatment for relapsed chronic myelogenous leukemia after allogeneic bone marrow transplantation: efficacy and toxicity of a defined T-cell dose. Blood. 1993;82:2310-2318.
12. Kolb HJ, Mittermuller J, Clemm C, et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. Blood. 1990;76:2462-2465.
13. Kolb HJ, Schattenberg A, Goldman JM, et al. Graft-versus-leukemia effect of donor lymphocyte transfusions in marrow grafted patients. Blood. 1995;86:2041-2050.
14. Mackinnon S, Papadopoulos EB, Carabasi MH, Reich L, Collins NH, O'Reilly RJ. Adoptive immunotherapy using donor leukocytes following bone marrow transplantation for chronic myeloid leukemia: is T cell dose important in determining biological response? Bone Marrow Transplant. 1995;15:591-594.
15. van Rhee F, Lin F, Cullis JO, et al. Relapse of chronic myeloid leukemia after allogeneic bone marrow transplant: the case for giving donor leukocyte transfusions before the onset of hematologic relapse. Blood. 1994;83:3377-3383.
16. Battiwalla M, Barrett J. Allogeneic transplantation using non-myeloablative transplant regimens. Best Pract Res Clin Haematol. 2001;14:701-722.
17. Giralt S. Reduced-Intensity Conditioning Regimens for Hematologic Malignancies: What Have We Learned over the Last 10 Years? Hematology Am Soc Hematol Educ Program. 2005:384-389.
18. McSweeney PA, Niederwieser D, Shizuru JA, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. Blood. 2001;97:3390-3400.
19. Sandmaier B, Storb R. Nonmyeloablative therapy and hematopoietic cell transplantation for hematologic disorders. (ed IIIrd). Oxford, U.K.: Blackwell Publishing Ltd; 2004.
20. Childs R, Clave E, Contentin N, et al. Engraftment kinetics after nonmyeloablative allogeneic peripheral blood stem cell transplantation: full donor T-cell chimerism precedes alloimmune responses. Blood. 1999;94:3234-3241.
21. Baron F, Sandmaier BM. Chimerism and outcomes after allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning. Leukemia. 2006;20:1690-1700.
22. Baron F, Little MT, Storb R. Kinetics of engraftment following allogeneic hematopoietic cell transplantation with reduced-intensity or nonmyeloablative conditioning. Blood Rev. 2005;19:153-164.

23. Sorror ML, Maris MB, Storer B, et al. Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplantation comorbidities. Blood. 2004;104:961-968.
24. Diaconescu R, Flowers CR, Storer B, et al. Morbidity and mortality with nonmyeloablative compared with myeloablative conditioning before hematopoietic cell transplantation from HLA-matched related donors. Blood. 2004;104:1550-1558.
25. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. Blood. 2005;106:2912-2919.
26. Bacigalupo A, Vitale V, Corvo R, et al. The combined effect of total body irradiation (TBI) and cyclosporin A (CyA) on the risk of relapse in patients with acute myeloid leukaemia undergoing allogeneic bone marrow transplantation. Br J Haematol. 2000;108:99-104.
27. Giralt S, Thall PF, Khouri I, et al. Melphalan and purine analog-containing preparative regimens: reduced-intensity conditioning for patients with hematologic malignancies undergoing allogeneic progenitor cell transplantation. Blood. 2001;97:631-637.
28. Gorin NC, Estey E, Jones RJ, Levitsky HI, Borrello I, Slavin S. New Developments in the Therapy of Acute Myelocytic Leukemia. Hematology (Am Soc Hematol Educ Program). 2000:69-89.
29. Shimoni A, Hardan I, Shem-Tov N, et al. Allogeneic hematopoietic stem-cell transplantation in AML and MDS using myeloablative versus reduced-intensity conditioning: the role of dose intensity. Leukemia. 2006;20:322-328.
30. Barrett J. Fludarabine finds its significant other? Blood. 2004;104:603-604.
31. Markova M, Barker JN, Miller JS et al. Fludarabine vs cladribine plus busulfan and low-dose TBI as reduced intensity conditioningfor allogeneic hematopoietic stem cell transplantation: a prospective randomized trial. Bone Marrow Transplantation. 2007; 39:193-199.
32. Takahata M, Hashino S, Okada K et al. Reduced intensity conditioning regimen with fludarabine, busulfan, and low-dose TBI (FLU-BU2-TBI): clinical efficacy in high-risk patients. Am J Hematol. 2010; 85:243-8.
33. Gandhi V, Plunkett W. Cellular and clinical pharmacology of fludarabine. Clin Pharmacokinet. 2002;41:93-103.
34. Belkacemi Y, Labopin M, Hennequin C, et al. Reduced-intensity conditioning regimen using low-dose total body irradiation before allogeneic transplant for hematologic malignancies: Experience from the European Group for Blood and Marrow Transplantation. Int J Radiat Oncol Biol Phys. 2007;67:544-551.
35. Mehta J, Powles R, Singhal S, Horton C, Tait D, Treleaven J. Melphalan-total body irradiation and autologous bone marrow transplantation for adult acute leukemia beyond first remission. Bone Marrow Transplant. 1996;18:119-123.
36. Abraham R, Chen C, Tsang R, et al. Intensification of the stem cell transplant induction regimen results in increased treatment-related mortality without improved outcome in multiple myeloma. Bone Marrow Transplant. 1999;24:1291-1297.
37. Camitta BM, Thomas ED, Nathan DG, et al. A prospective study of androgens and bone marrow transplantation for treatment of severe aplastic anemia. Blood. 1979;53:504-514.
38. Locasciulli A, Oneto R, Bacigalupo A, et al. Outcome of patients with acquired aplastic anemia given first line bone marrow transplantation or immunosuppressive treatment in the last decade: a report from the European Group for Blood and Marrow Transplantation (EBMT). Haematologica. 2007;92:11-18.
39. Goi K, Sugita K, Nakamura M, et al. Natural pregnancy and delivery after allogeneic bone marrow transplantation in a Fanconi anaemia patient. Br J Haematol. 2006;135:410-411.
40. Zanis-Neto J, Flowers ME, Medeiros CR, et al. Low-dose cyclophosphamide conditioning for haematopoietic cell transplantation from HLA-matched related donors in patients with Fanconi anaemia. Br J Haematol. 2005;130:99-106.
41. George B, Mathews V, Shaji RV, Srivastava V, Srivastava A, Chandy M. Fludarabine-based conditioning for allogeneic stem cell transplantation for multiply transfused patients with Fanconi's anemia. Bone Marrow Transplant. 2005;35:341-343.
42. Srinivasan R, Takahashi Y, McCoy JP, et al. Overcoming graft rejection in heavily transfused and allo-immunised patients with bone marrow failure syndromes using fludarabine-based haematopoietic cell transplantation. Br J Haematol. 2006;133:305-314.

43. Takahashi Y, McCoy JP, Jr., Carvallo C, et al. In vitro and in vivo evidence of PNH cell sensitivity to immune attack after nonmyeloablative allogeneic hematopoietic cell transplantation. Blood. 2004;103:1383-1390.
44. Mayer RJ, Davis RB, Schiffer CA, et al. Intensive postremission chemotherapy in adults with acute myeloid leukemia. Cancer and Leukemia Group B. N Engl J Med. 1994;331:896-903.
45. Grimwade D, Walker H, Harrison G, et al. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. Blood. 2001;98:1312-1320.
46. Leith CP, Kopecky KJ, Godwin J, et al. Acute myeloid leukemia in the elderly: assessment of multidrug resistance (MDR1) and cytogenetics distinguishes biologic subgroups with remarkably distinct responses to standard chemotherapy. A Southwest Oncology Group study. Blood. 1997;89:3323-3329.
47. Wheatley K, Burnett AK, Goldstone AH, et al. A simple, robust, validated and highly predictive index for the determination of risk-directed therapy in acute myeloid leukaemia derived from the MRC AML 10 trial. United Kingdom Medical Research Council's Adult and Childhood Leukaemia Working Parties. Br J Haematol. 1999;107:69-79.
48. Alyea EP, Kim HT, Ho V, et al. Comparative outcome of nonmyeloablative and myeloablative allogeneic hematopoietic cell transplantation for patients older than 50 years of age. Blood. 2005;105:1810-1814.
49. Scott BL, Sandmaier BM, Storer B, et al. Myeloablative vs nonmyeloablative allogeneic transplantation for patients with myelodysplastic syndrome or acute myelogenous leukemia with multilineage dysplasia: a retrospective analysis. Leukemia. 2006;20:128-135.
50. Cutler CS, Lee SJ, Greenberg P, et al. A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: delayed transplantation for low-risk myelodysplasia is associated with improved outcome. Blood. 2004;104:579-585.
51. Barosi G, Hoffman R. Idiopathic myelofibrosis. Semin Hematol. 2005;42:248-258.
52. Hoffman R, Prchal JT, Samuelson S, Ciurea SO, Rondelli D. Philadelphia chromosome-negative myeloproliferative disorders: biology and treatment. Biol Blood Marrow Transplant. 2007;13 Suppl 1:64-72.
53. Rondelli D, Barosi G, Bacigalupo A, et al. Allogeneic hematopoietic stem-cell transplantation with reduced-intensity conditioning in intermediate- or high-risk patients with myelofibrosis with myeloid metaplasia. Blood. 2005;105:4115-4119.
54. Khouri IF. Reduced-intensity regimens in allogeneic stem-cell transplantation for non-hodgkin lymphoma and chronic lymphocytic leukemia. Hematology Am Soc Hematol Educ Program. 2006:390-397.
55. Rodriguez R, Nademanee A, Ruel N, et al. Comparison of reduced-intensity and conventional myeloablative regimens for allogeneic transplantation in non-Hodgkin lymphoma. Biol Blood Marrow Transplant. 2006;12:1326-1334.
56. Sorror ML, Maris MB, Sandmaier BM, et al. Hematopoietic cell transplantation after nonmyeloablative conditioning for advanced chronic lymphocytic leukemia. J Clin Oncol. 2005;23:3819-3829.
57. Peggs KS, Hunter A, Chopra R, et al. Clinical evidence of a graft-versus-Hodgkin-lymphoma effect after reduced-intensity allogeneic transplantation. Lancet. 2005;365:1934-1941.
58. Barlogie B, Kyle RA, Anderson KC, et al. Standard chemotherapy compared with high-dose chemoradiotherapy for multiple myeloma: final results of phase III US Intergroup Trial S9321. J Clin Oncol. 2006;24:929-936.
59. Bensinger WI, Maloney D, Storb R. Allogeneic hematopoietic cell transplantation for multiple myeloma. Semin Hematol. 2001;38:243-249.
60. Badros A, Barlogie B, Siegel E, et al. Improved outcome of allogeneic transplantation in high-risk multiple myeloma patients after nonmyeloablative conditioning. J Clin Oncol. 2002;20:1295-1303.
61. Kroger N, Sayer HG, Schwerdtfeger R, et al. Unrelated stem cell transplantation in multiple myeloma after a reduced-intensity conditioning with pretransplantation antithymocyte globulin is highly effective with low transplantation-related mortality. Blood. 2002;100:3919-3924.
62. Maloney DG, Molina AJ, Sahebi F, et al. Allografting with nonmyeloablative conditioning following cytoreductive autografts for the treatment of patients with multiple myeloma. Blood. 2003;102:3447-3454.
63. Bruno B, Rotta M, Patriarca F. A Comparison of Allografting with Autografting for Newly Diagnosed Myeloma. NEJM. 2007;356:1110-1120.

64. Uzunel M, Remberger M, Sairafi D, et al. Unrelated versus related allogeneic stem cell transplantation after reduced intensity conditioning. Transplantation. 2006;82:913-919.
65. Clift RA, Buckner CD, Appelbaum FR, et al. Allogeneic marrow transplantation during untreated first relapse of acute myeloid leukemia. J Clin Oncol. 1992;10:1723-1729.
66. Gupta V, Yi QL, Brandwein J, et al. The role of allogeneic bone marrow transplantation in adult patients below the age of 55 years with acute lymphoblastic leukemia in first complete remission: a donor vs no donor comparison. Bone Marrow Transplant. 2004;33:397-404.
67. Sebban C, Lepage E, Vernant JP, et al. Allogeneic bone marrow transplantation in adult acute lymphoblastic leukemia in first complete remission: a comparative study. French Group of Therapy of Adult Acute Lymphoblastic Leukemia. J Clin Oncol. 1994;12:2580-2587.
68. Sutton L, Kuentz M, Cordonnier C, et al. Allogeneic bone marrow transplantation for adult acute lymphoblastic leukemia in first complete remission: factors predictive of transplant-related mortality and influence of total body irradiation modalities. Bone Marrow Transplant. 1993;12:583-589.
69. Barrett AJ, Horowitz MM, Pollock BH, et al. Bone marrow transplants from HLA-identical siblings as compared with chemotherapy for children with acute lymphoblastic leukemia in a second remission. N Engl J Med. 1994;331:1253-1258.
70. Fielding AK, Richards SM, Chopra R, et al. Outcome of 609 adults after relapse of acute lymphoblastic leukemia (ALL); an MRC UKALL12/ECOG 2993 study. Blood. 2007;109:944-950.
71. Schlenk RF, Corbacioglu A, Krauter J, et al. Gene Mutations as Predicitive Markers for Postremission Therapy in Younger Adults with Normal Karyotype AML. ASH Annual Meeting Abstracts. 2006;108:4-.
72. Moorman AV, Harrison CJ, Buck GA, et al. Karyotype is an independent prognostic factor in adult acute lymphoblastic leukemia (ALL): Analysis of cytogenetic data from patients treated on the Medical Research Council (MRC) UKALLXII / Eastern Cooperative Oncology Group (ECOG) 2993 Trial. Blood. 2006.
73. Rowe JM, Buck G, Fielding A, et al. In Adults with Standard-Risk Acute Lymphoblastic Leukemia (ALL) the Greatest Benefit Is Achieved from an Allogeneic Transplant in First Complete Remission (CR) and an Autologous Transplant Is Less Effective Than Conventional Consolidation/Maintenance Chemotherapy: Final Results of the International ALL Trial (MRC UKALL XII/ECOG E2993). ASH Annual Meeting Abstracts. 2006;108:2-.
74. Hertzberg M, Grigg A, Gottlieb D, et al. Reduced-intensity allogeneic haemopoietic stem cell transplantation induces durable responses in patients with chronic B-lymphoproliferative disorders. Bone Marrow Transplant. 2006;37:923-928.
75. Kiss TL, Mollee P, Lazarus HM, Lipton JH. Stem cell transplantation for mantle cell lymphoma: if, when and how? Bone Marrow Transplant. 2005;36:655-661.
76. van Besien KW, de Lima M, Giralt SA, et al. Management of lymphoma recurrence after allogeneic transplantation: the relevance of graft-versus-lymphoma effect. Bone Marrow Transplant. 1997;19:977-982.
77. Flomenberg N, Baxter-Lowe LA, Confer D, et al. Impact of HLA class I and class II high-resolution matching on outcomes of unrelated donor bone marrow transplantation: HLA-C mismatching is associated with a strong adverse effect on transplantation outcome. Blood. 2004;104:1923-1930.
78. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. Blood. 2000;96:2062-2068.
79. Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. Blood. 1998;92:2303-2314.
80. Maris MB, Sandmaier BM, Storer BE, et al. Unrelated donor granulocyte colony-stimulating factor-mobilized peripheral blood mononuclear cell transplantation after nonmyeloablative conditioning: the effect of postgrafting mycophenolate mofetil dosing. Biol Blood Marrow Transplant. 2006;12:454-465.
81. Luisa Giaccone, Jeannine S. McCune, Michael B. Maris . Pharmacodynamics of mycophenolate mofetil after nonmyeloablative conditioning and unrelated donor hematopoietic cell transplantation. Blood, 2005; 106,13, 4381-4388.
82. A Elefante1, J Muindi, K West, et. al, Long-term stability of a patient-convenient 1 mg/ml suspension of tacrolimus for accurate maintenance of stable therapeutic levels. Bone Marrow Transplantation 2006: 37, 781–784

83. Baron F., Storb R. Allogeneic hematopoietic cell transplantation following nonmyeloablative conditioning as treatment for hematologic malignancies and inherited blood disorders. Mol Ther. 2006;13:26-41.
84. Haentzschel I, Freiberg-Richter J, Platzbecker U, et al. Targeting mycophenolate mofetil for graft-versus-host disease prophylaxis after allogeneic blood stem cell transplantation. 2008:42:113-20

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 PO2 (>10% from baseline) but not requiring mechanical ventilation or > 50% O2 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 Sl et al. Regimen Related Toxicity in Patients Undergoing Bone Marrow Transplantation. JCO 1988, 6(10); 1562-15*

Appendix No. II

WHO ORAL MUCOSITIS GRADING

Grade 0	No changes
Grade 1	Soreness/erythema
Grade 2	Soreness/erythema + ulceration + can eat solid foods
Grade 3	Soreness/erythema + ulceration + can use a liquid diet only
Grade 4	Soreness/erythema + ulceration + oral alimentation is not possible

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

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.

Clinical impression of overall chronic GvHD severity will be described as: mild, moderate and severe.

1. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man: a long-term clinicopathologic study of 20 Seattle patients. Am J Med. 1980;69:204-217

2. Lee SJ, Klein JP, Barrett AJ, et al. Severity of chronic graft-versus-host disease: association with treatment-related mortality and relapse. Blood. 2002;100: 406-414

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 under the supervision of the attending physician. 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 through a wide lumen central catheter. No more than 480 ml of stem cells may be infused at one time and the transplant may need to be split into a morning and afternoon infusion at the discretion of the attending physician. Patients will be pre-medicated according to RPCI Standard Operating Procedure for Stem Cell reinfusion.

Appendix No. VI

VI A. 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

Karnofsky DA, Burchenal JH. The Clinical Evaluation of Chemotherapeutic Agents in Cancer. In: MacLeod CM (Ed), "Evaluation of Chemotherapeutic Agents." Columbia Univ Press, 1949:196.

VI B. Lansky Performance Status (16 years and younger)

- 100 - fully active, normal
- 90 - minor restrictions in strenuous physical activity
- 80 - active, but tired more quickly
- 70 - greater restriction of play *and* less time spent in play activity
- 60 - up and around, but active play minimal; keeps busy by being involved in quieter activities
- 50 - lying around much of the day, but gets dressed; no active playing participates in all quiet play and activities
- 40 - mainly in bed; participates in quiet activities
- 30 - bedbound; needing assistance even for quiet play
- 20 - sleeping often; play entirely limited to very passive activities
- 10 - doesn't play; does not get out of bed
- 0 - unresponsive

Lansky SB, List MA, Lansky LL, Ritter-Sterr C, Miller DR. The measurement of performance in childhood cancer patients. Cancer 1987;60:1651-6

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

COCKROFT GAULT FORMULA (MODIFIED FOR BSA) FOR ESTIMATION OF CREATININE CLEARANCE ABOVE AGE 18

CLEARANCE (ml/min)
Male = (140-age) x IBW (kg) / 72 x SrCr
Female= male clearance x 0.85

IBW (male) = 50 + 0.91(Height(cm) - 152)
IBW (female) = 45 + 0.91(Height (cm) - 152)
SrCr in mg/dL.

SCHWARTZ FORMULA FOR ESTIMATION OF CREATININE CLEARANCE UP TO AGE 18

CLEARANCE (ml/min/1.73 m2) = K x L / SrCr

K = age adjusted constant, L = length in centimeters, SrCr in mg/dL.

Age	K
2-12 y	0.55
13-21 y female	0.55
13-21 y male	0.70

Appendix No. VIII

Tests/Observations*	Pre-Rx	Post-Rx Δ
History and physical exam, Comorbidity Index	x	--
Signed Informed Consent	x	--
Ht/Wt/ BSA	x	--
Serology (HIV, Hepatitis, CMV)	x	--
HLA typing	x	--
Cr Clearance	x	--
PFT's including DLCO	x	At day 100, then as indicated [+/- 7 days] €
MUGA or ECHO	x	--
EKG	x	--
Quantitative Immunoglobulins	x	X†
BM aspirate, biopsy §	x	day 30, day 100 and then as indicated [+/-7 days] €
Peripheral blood for chimerism	x	day 30, day 100 and then as indicated [+/-7 days] €
Molecular, flow and cytogenetics analysis	x	day 30, day 100, 1 year and then as indicated [+/-7 days] €
CBC/diff	x	q day till PMN≥500, Plt≥20K, then as indicated
Urinalysis biochemical	x	as clinically indicated
PT	x	as clinically indicated
Comprehensive metabolic panel, LDH	x	q wk x 4 and then as indicated
Chest X-ray (PA and lateral)	x	--
CT Scan and/or PET ††	x	At day 100 and then as indicated [+/-7 days] €
Karnofsky/Lansky Performance Status	x	q wk x 4 and then as indicated
Toxicity (Bearman/WHO mucositis)	--	wkly x 4 and then day 100 & then as indicated
Acute GVHD assessment	--	q wk till day +100
Chronic GVHD assessment	--	as indicated

* 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.

† Post transplant SPEP, IFE, Quantitative immunoglobulins, serum beta-2 microglobulin, kappa/lambda free light chains and 24 hour urine for protein electrophoresis and free light chains will be done only for patients with multiple myeloma on day 100-160 and then as clinically indicated.

§ BM aspirate and biopsy will be performed for patients with leukemias and bone marrow failure states 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.

†† For patients with lymphoma only. Also palpable tumor measurement should be recorded for lymphoma patients.

Δ All testing will be performed post-Rx as clinically indicated. Only mandatory tests are included in this table.

€ If the patient is medically unfit or has scheduling difficulty, delay is allowed.