FRED HUTCHINSON CANCER RESEARCH CENTER
UNIVERSITY OF WASHINGTON SCHOOL OF MEDICINE
SEATTLE CHILDREN'S

A Multi-center Phase II Study of Selective Depletion of CD45RA+ T Cells from Allogeneic Peripheral Blood Stem Cell Grafts for the Prevention of GVHD

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1. Title of Protocol: A Multi-center Phase II Study of Selective Depletion of CD45RA$^+$ T Cells from Allogeneic Peripheral Blood Stem Cell Grafts for the Prevention of GVHD

Coordinating Site:

Investigator	**Professional Title**	**Telephone number**
Marie Bleakley, MD, PhD (PI)	Assistant Member FHCRC Assistant Professor of Pediatrics, UW	(206) 667-6572 (206) 469-4487 (pager)
Stanley Riddell, MD	Member, FHCRC Professor of Medicine, UW	(206) 667-5249
Shelly Heimfeld, PhD	Shelly Heimfeld, PhD Member, FHCRC	(206) 667-4004
Biostatistician		
Ted Gooley, PhD	Associate Member, FHCRC Affiliate Associate Professor, UW	(206) 667-6533
Radiation Oncologist		
Ralph Ermoian, MD	Assistant Professor of Radiation Oncology, UW	(206) 598-4100
Research Staff		
Colette Chaney, RN	Research Nurse	(206) 667-5823 (206) 559-4104 (pager) (206) 667-6056 (fax)

Participating Sites:

Yale University School of Medicine/Yale New Haven Hospital		
Investigator & IDE Sponsor		
Warren Shlomchik, MD	Professor of Medicine, Yale University School of Medicine	(203) 737-2478
Co-Investigators		
Kenneth Roberts, MD	Associate Professor in Therapeutic Radiology, Yale University School of Medicine	(203) 785-2957
Stuart Seropian, MD	Associate Professor of Medicine, Yale University School of Medicine	(203) 737-5751
Dennis Cooper, MD	Professor of Medicine, Yale University School of Medicine	(203) 737-5751

Emergency (24 hour) Phone: (206) 598-8902; **Fax:** (206) 598-4034

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2. Introduction

Graft versus host disease (GVHD) is a frequent complication of allogeneic hematopoietic cell transplantation (HCT). Morbidity and mortality as a consequence of GVHD result from direct organ damage, and from infections and organ toxicity related to the use of immunosuppressive drugs to treat GVHD [1]. Despite the administration of immunosuppressive drugs early post transplant to prevent GVHD, the incidence of grade II – IV acute GVHD following allogeneic peripheral blood stem cell (PBSC) grafts from human leukocyte antigen (HLA) matched sibling donors at the FHCRC and other centers is 40-80% [2-4]. In HCT using HLA-identical sibling donors, GVHD results from recognition of minor histocompatibility (H) antigens expressed on recipient tissues by donor T cells [5]. Complete depletion of T cells from the donor hematopoietic cell product is a highly effective alternative to pharmacologic immunosuppression for preventing GVHD, but is complicated by a profound delay in immune reconstitution, which contributes to life threatening infections, and has been associated with an increased risk of graft rejection and leukemia relapse [6, 7]. Laboratory studies have suggested that donor T cells specific for recipient minor H antigens are found predominantly within the naïve subset of T cells that express the cell surface molecule CD45RA, and that the selective depletion of $CD45RA^+$ T cells from peripheral blood stem cell (PBSC) grafts can preserve the subset of pathogen-specific memory T cells that lack CD45RA.This protocol will evaluate the selective removal of $CD45RA^+$ cells from PBSC as a strategy to reduce the rate of grades II-IV acute GVHD and provide rapid recovery of T cell immunity to pathogens after HCT from HLA-identical sibling donors.

3. Background

3A. GVHD

The pathogenesis of GVHD involves multiple interacting factors including tissue damage resulting from the conditioning regimen, the release of inflammatory cytokines, and the activation and proliferation of mature donor T cells that express the αβ T cell receptor (TCR) and recognize recipient alloantigens presented as peptides by class I and II major histocompatibility complex (MHC or HLA in humans) molecules on antigen presenting cells (APCs) [1]. The mature T cell repertoire is "tolerant" to peptides derived from self-proteins due to thymic deletion and/or peripheral suppression of autoreactive T cells [8]. However, in the setting of allogeneic HCT between HLA-identical individuals, the repertoire of peptides displayed on recipient cells will include distinct species that differ from those on donor cells as a consequence of polymorphisms in genes outside of the MHC, and these peptides can be recognized as minor H antigens by donor T cells [5]. The activation of donor T cells to recipient minor H antigens is likely to occur after contact with specialized APCs and leads to proliferation of T cells and their differentiation to effector cells that produce cytokines including IFN-γ and TNF, and mediate cytotoxicity against recipient tissues [5]. The tissues that are most frequently damaged during acute GVHD include skin, gastrointestinal tract, and liver, although other organs can be involved, particularly when acute GVHD evolves into chronic GVHD.

The administration of immunosuppressive drugs that interfere with T cell activation or proliferation such as methotrexate, cyclosporine, FK506, prednisone, and mycophenolate mofetil, alone or in various combinations, is used at most transplant centers to prevent GVHD [9]. The development in the early 1980s of the combination of a short course of methotrexate with the calcineurin inhibitor cyclosporine represented a significant

advance in the prophylaxis of GVHD, but major improvements in pharmacologic immunosuppression have not been achieved since that time [9, 10]. At the FHCRC, a short course of methotrexate combined with either cyclosporine or FK506 continues to be the most frequently used immunosuppressive regimen for GVHD prophylaxis after allogeneic HCT. With this regimen of GVHD prophylaxis, the incidence of grade II-IV acute GVHD was 61% in patients with acute leukemia that had a HCT from an HLA-identical sibling donor at the FHCRC since 2003. Patients that develop acute GVHD require treatment with additional immunosuppressive drugs, which increases their risk of post transplant infections and may diminish the graft versus leukemia (GVL) effect [11]. Additionally, many patients with acute leukemia will relapse after HCT and those with GVHD are less likely to be eligible for, or benefit from, T cell immunotherapy designed to augment the GVL effect.

3B. T Cell Depletion to Prevent GVHD

The recognition of recipient minor H antigens by donor T cells is an essential requirement for the induction of GVHD after HCT from HLA-identical sibling donors. Thus, many centers have used partial or complete depletion of T cells from the donor bone marrow or PBSC graft as an alternative approach to pharmacologic immunosuppression for preventing GVHD [6, 7, 12]. A variety of methods have been used to remove T cells from bone marrow or peripheral blood stem cell (PBSC) grafts including soybean lectin agglutination, monoclonal antibodies, and positive selection of $CD34^+$ hematopoietic progenitors [6, 7, 12]. Depending on the approach, the extent of T cell depletion can vary from 2-5 $\log_{10}$ More complete T cell depletion is generally associated with less GVHD, although an absolute threshold dose of donor T cells for GVHD has been difficult to define in humans.

T cell depletion is a highly effective strategy for preventing GVHD in both murine models and in humans [6, 7, 12, 13], but recipients of T-cell depleted grafts have poor reconstitution of T cell immunity to pathogens, and an increased risk of graft rejection and relapse [6, 7, 12-14]. A Phase 2 study at Memorial Sloan Kettering Cancer Center (MSKCC) used soybean lectin agglutination and sheep red blood cell rosette depletion to remove T cells from donor bone marrow, and administered an intensified pretransplant conditioning regimen that included total body irradiation (TBI --15 Gy with lung shielding to 8 – 9 Gy), cyclophosphamide (Cy), and thiotepa to patients receiving allogeneic HLA-identical HCT for AML in remission. ATG was also administered pre and post transplant to prevent graft rejection. Graft rejection occurred in 0 of 39 patients in the MSKCC study [12]. The toxicity with this conditioning regimen was tolerable with 2 deaths in the 39 patients due to veno-occlusive disease and interstitial pneumonia, respectively. The risk of Epstein-Barr virus lymphoproliferative disease (EBV-LPD) was ~10% in the MSKCC studies and 6/39 patients died of infectious complications (3 – cytomegalovirus (CMV) pneumonia, 1 - toxoplasmosis, 1 – pneumocystis carinii, 1 fungal pneumonia). The relapse rates for AML patients in 1^{st} and 2^{nd} complete remission at the time of transplant in the MSKCC study were 3.2% and 12.5% respectively. The probability of disease free survival for patients transplanted in 1^{st} CR was 77.4% at 4 years, and for patients transplanted in 2^{nd} CR was 50% at 3 years [12]. The MSKCC results are comparable to the results achieved with unmodified PBSC grafts at the FHCRC and other centers [2]. Based on these results, the Bone Marrow Transplant Clinical Trials Network is currently conducting a multicenter trial of complete T cell depletion for AML patients undergoing HCT from HLA-identical sibling donors. The

BMT-CTN study is using TBI (13.75 Gy), Cy, and thiotepa as a conditioning regimen, and the Miltenyi CliniMACS device to select $CD34^+$cells from G-CSF mobilized PBSC using anti CD34 coated immunomagnetic beads. CD34 selection with the Miltenyi CliniMACS device will provide a 4-5 $\log_{10}$ removal of T cells including memory T cells, and delayed recovery of T-cell immunity and opportunistic infections are expected to be significant obstacles to a successful outcome for these patients.

Recently, the results of 52 patients (age 19.5 – 62 years) with various hematologic malignancies treated at MSKCC with T cell depleted HCT and a conditioning regimen consisting of fludarabine (Flu), thiotepa, and TBI (13.75 Gy) without ATG were reported. This regimen was well tolerated with a 100 day mortality of ~10%. There were no graft rejections in this study although two patients received donor lymphocyte infusion for mixed chimerism. The rate of acute GVHD was 8%, and the disease free survival was 72% for patients with standard risk disease and 52% for patients with poor risk disease at a median follow-up of 45 months [13]. The cause specific probability of relapse for all patients was 17%. Detailed studies of immune reconstitution in these patients were not reported, however eight of 52 patients died from infection, and there were two cases of EBV-LPD [13]. These findings demonstrate that ATG is not required with this conditioning regimen to prevent graft rejection after T cell depleted HCT, and suggest that the substitution of Flu for Cy in the regimen may reduce toxicity.

3C. Naive T cells (T_N) are primarily responsible for GVHD in rodent models

Transplant approaches that selectively deplete T cells that recognize minor H antigens and retain T cells specific for pathogens in the stem cell graft could reduce GVHD and improve reconstitution of T cell immunity. Recent studies in murine models of allogeneic stem cell transplantation have provided insights into how such selective manipulation of the T cell content of hematopoietic cell grafts might be achieved. Mature $CD3^+$ $CD8^+$ and $CD3^+$ $CD4^+$ T cells can be classified into naïve (T_N) and memory (T_M) subsets that differ in cell surface phenotype, prior exposure to cognate antigen, and functional activity [15, 16]. In mice, the T_N subset is $CD44^-$ $CD62L^+$ and the T_M subset is $CD44^+$ and can be further subdivided into a $CD62L^+$ central memory (T_{CM}) population, and a $CD62L^-$ effector memory (T_{EM}) population [16]. Studies in murine bone marrow transplant models have evaluated the potential for T cells derived from T_N and T_M subsets to cause GVHD across both minor and major histocompatiblity differences. The first study to examine this question employed a multiple minor H antigen mismatched $CD4^+$ dependent GVHD model [B10.D2 ($H\text{-}2^d$) → BALB/c ($H\text{-}2^d$)]. In this model, transplantation of irradiated BALB/c mice with T-cell depleted bone marrow combined with unfractionated splenocytes or with purified $CD4^+$ $CD44^-$ $CD62L^+$ T_N from B10.D2 donors caused severe GVHD. However, transplantation of T-cell depleted bone marrow with purified $CD4^+$ $CD44^+$ $CD62L^-$ T_M did not cause GVHD and provided for the transfer of T cell immunity to a model antigen [17].

The efficacy of removing T_N from the stem cell graft for preventing GVHD has been confirmed in other murine strain combinations including CD8 dependent minor H antigen-mismatched and MHC-mismatched models, and in rats [17-22][Table 1]. It should be noted that the intent in these studies was to deplete T_N, but the cell selection procedure that was utilized in some studies would also remove the T_{CM} subset of T_M from the graft. In other studies, the selection procedure targeting $CD44^+$ did not allow the effects of T_{CM} or T_{EM} to be delineated. In subsequent experiments by the Shlomchik

group, purified T_N or T_{CM} obtained by cell sorting were transplanted with T cell depleted bone marrow in a CD8 dependent minor H antigen-mismatched murine model of GVHD in the absence of immunosuppression. Mice that received T_N developed severe GVHD, whereas mice that received T_{CM} developed only mild GVHD [19]. Similar results were obtained in an MHC-mismatched model. These results demonstrate that in murine allogeneic minor H antigen and MHC mismatched bone marrow transplantation, the T_N subset of T cells is primarily responsible for GVHD and bone marrow grafts containing only T_M exhibit limited capacity to cause GVHD.

Table 1. Rodent studies of T cell depleted BMT with selective addition of T cell subsets

Transplant Model	Study	T Cell Subsets	Experimental Design	Outcome
Multiple Minor H Antigen Mismatched, CD4 dependent	Anderson B et al. J Clin Invest, 112: 101, 2003 [17]	$CD4^+$ $CD44^+$ $CD62L^-$ (T_M) vs $CD4^+$ $CD44^-$ $CD62L^+$ (T_N)	B10.D2 (H-2^d) →BALB/c (H-2^d)	No GVHD in mice receiving T_M, severe GVHD in mice receiving T_N
Multiple Minor H Antigen Mismatched, CD8 dependent	Zhang Y. et al. Blood 103; 3970 – 3978, 2004 [18]	$CD8^+$ $CD44^+$(T_M) vs $CD8^+$ $CD44^-$ (T_N)	CH3.SW (H-2^b) →B6 (H-2^b)	Minimal GVHD in mice receiving T_M, severe GVHD in mice receiving T_N
Multiple Minor H Antigen Mismatched CD8 dependent	Zheng H et al. Blood 106 (abstract 1312), 2005 [19]	$CD8^+CD44^+CD62L^+$(T_{CM}) vs $CD8^+$ $CD44^-$ $CD62L^+$(T_N)	CH3.SW (H-2^b) →B6 (H-2^b)	Mild GVHD in mice receiving T_{CM}, severe GVHD in mice receiving T_N
MHC Mismatched	Chen B et al. Blood 103: 1534, 2004 [20]	$CD4^+$ + $CD8^+$ $CD62L^-$ (T_{EM}) vs $CD4^+$ + $CD8^+$ $CD62L^+$ (T_N and T_{CM})	C57BL/6 (H-2^b) → BALB/c (H-2^d) or C3H/HeJ (H-2^k)	No GVHD in mice that received $CD62L^-$ T cells, lethal GVHD in recipients of $CD62L^+$ T cells
MHC Mismatched	Zheng et al, Blood, 2008.111(4): 2476 -84.[21]	$CD4^+CD44^+CD62L^-$(T_{EM}) vs CD4+CD44-CD62L-(T_N)	$B6^{bm12}$→B6	No GVHD in T_{EM} recipients; GVHD in T_N recipients.
MHC Mismatched (Rat Model)	Xystrakis E et al. Eur J Immunol, 34: 408, 2004 [22]	$CD4^+$ $CD45RC^{high}$ (T_N) vs $CD4^+$ $CD45RC^{low}$ (T_M)	Parental →F1 (LEWxBN)	No GVHD in rats receiving T_M, lethal GVHD in rats receiving T_N

3D. Human T cells specific for minor H antigens are predominantly in the T_N subset. Inferential data suggests that the results obtained in murine models using selective depletion of T_N to prevent GVHD might also apply to humans. The T_N subset in humans, which expresses both CD45RA and CD62L, has been shown to contain more than 99% of the overall T cell receptor (TCR) diversity [23]. The T_M subset, which contains $CD45RO^+$ $CD62L^+$ T_{CM} and $CD62L^-$ T_{EM} populations, and a subset of $CD62L^-$ cells that re-express CD45RA (T_{EMRA}), is comprised of cells that have clonally expanded in response to antigen stimulation and contains <1% of the overall TCR diversity. A major fraction of the T_M subset consists of T cells specific for latent herpes viruses including CMV, EBV, herpes simplex virus (HSV) and varicella zoster virus (VZV). Cross reactivity of virus-specific T_M for major alloantigens has been observed only rarely [24] , and cross reactivity with minor H antigens has not been reported and is likely to be exceedingly rare. To evaluate the presence of minor H antigen-reactive T cells in T_N and

T_M subsets, we purified $CD8^+$ T_N ($CD45RA^{bright}$, $CD62L^+$, $CD45RO^-$) and T_M ($CD45RO^+$, $CD62L^{+/-}$, $CD45RA^-$) from leukapheresis products obtained from HLA-identical sibling pairs and determined the frequency of alloreactive T cells in each subset by limiting dilution analysis. Individuals may be exposed to minor H antigens by pregnancy or blood transfusion and develop minor H antigen-specific T_M responses, therefore donors without a prior history of pregnancy or blood transfusion were selected for these experiments. In multiple assays of different HLA-matched pairs, the frequency of minor H antigen-reactive T-cells was >3 $\log_{10}$ higher in the T_N subset than in the T_M subset. Indeed, only very rare cells (~1/10,000,000) in the T_M subset exhibited weak recognition of recipient cells in these assays, and this weak alloreactivity was not reproducible with repeated testing. Thus, in individuals without a history of pregnancy or blood transfusion, minor H antigen-specific $CD8^+$ T cells are predominantly in the T_N subset [25]. We attempted to evaluate the frequency of T cells specific for minor H antigens in $CD4^+$ T_N and T_M subsets using lymphoproliferation assays, but these assays were confounded by high background reactivity to autologous cells in both subsets.

3E. Depletion of T_N from human PBSC grafts as a strategy to reduce GVHD

The in vivo data in animal models and in vitro analysis of alloreactivity of $CD8^+$ T_M and T_N subsets in HLA-identical siblings suggest that selective depletion of T_N from human PBSC grafts is a rational strategy to investigate for reducing GVHD and for improving the tempo of immune reconstitution after allogeneic HCT. In preclinical studies, we have examined methods for selectively depleting T_N from G-CSF mobilized PBSC products. We initially considered using anti-CD62L mAb to deplete T_N, based on the data from murine models. However, CD62L is subject to proteolytic cleavage by ADAM-17 and other proteases released during G-CSF mobilization [26], and we found that cell surface CD62L declined on T cells in G-CSF mobilized products. CD45RA is expressed stably on all $CD8^+$ and $CD4^+$ T_N in G-CSF mobilized PBSC but a minor subset of $CD34^+$ cells express CD45RA. Thus, to remove $CD45RA^+$ T cells without interfering with $CD34^+$ progenitors, we used sequential positive selection of $CD34^+$ progenitor cells using Miltenyi CD34 immunomagnetic selection, followed by depletion of $CD45RA^+$ cells from the CD34-negative fraction. Our goal was to develop a cell selection procedure that would result in an engineered stem cell graft that contains >2.0 x 10^6 $CD34^+$ cells/kg recipient body weight, <7.5 x 10^4 T_N/kg and 1-10 x 10^6 T_M/kg. The dose of 1-10 x 10^6 $CD3^+$ cells/kg was selected for two reasons. First, this number is nearly equivalent to the T cell content of an unmanipulated bone marrow graft and is 10 - 100 fold greater than the threshold dose of unselected T cells that causes GVHD after HLA-identical sibling HCT [27]. It will therefore allow us to test the hypothesis that $CD45RA^-$ T cells can safely be transferred with less GVHD. Second, it will provide a sufficient number of memory T cells such that we would reasonably anticipate improved immune reconstitution as compared to recipients of only a TCD allograft.

$CD34^+$ selection consistently yields an enriched progenitor population with an extremely low residual T cell content and preserves the $CD34^+$ cells needed for engraftment, thereby negating any potential detrimental effects of the T cell manipulation on hematopoietic progenitors (S. Heimfeld, personal communication). In 41 $CD34^+$ selections at the FHCRC the range log T cell depletion was 4.2 to 5.5. Based on a starting T cell content of G-CSF mobilized products of 2 x 10^7 to 1 x 10^9/kg recipient body weight this depletion would result in a residual T cell content of the CD34-positive fraction of <= 6 x10^4 total $CD3^+$/kg of which 30-70% of the cells will be $CD45RA^+$. The

minimum yield of $CD34^+$ cells after $CD34^+$ selection is 40% therefore only G-CSF mobilized products where at least $5x10^6$ $CD34^+$ cells/kg of recipient body weight are obtained will be processed to ensure that the $CD34^+$ cell dose will be $\geq 2x10^6$ $CD34^+$ cells/kg of recipient body weight. Experience at FHCRC indicates that the products from 85% of donors will contain $\geq 5x10^6$ $CD34^+$ cells/kg of recipient body weight. The CD34 content of the GCSF mobilized products may potentially be increased further by administering GCSF to donors twice daily and a dosing schedule of GCSF 8mcg/kg bid will be employed in this protocol. Therefore, it is expected that cell selection will be performed for at least 85-90% of the enrolled patients.

A GMP-grade murine αCD45RA monoclonal antibody that is directly conjugated to Miltenyi iron dextran beads has been produced by Miltenyi Biotec under contract from the NIH RAID program. Our experience to date both from several mid scale depletions of $CD45RA^+$ T cells and from full scale depletions on the CliniMACs device indicates that the goal of obtaining a total T cell dose of $1\text{-}10x\ 10^6$

Table 2: Result of Large Scale CD45RA Depletions on GCSF-mobilized Apheresis products

	Unmanipulated PBSC (cells/kg)	CD45RA depleted PBSC (adjusted to 5×10^6 CD3 cells/kg)
Total TNC	2.3×10^8	1.5×10^7
Total $CD3^+$ T cells	1.03×10^8	5×10^6
$CD3^+$ $CD45RA^+$ (Naïve)	5.8×10^7	9.5×10^3 (8.8×10^2 -2×10^4)
$CD3^+$ $CD45RA^-$ (Memory)	4.5×10^7	5×10^6
$CD16^+$ NK cells	1.7×10^7	1.2×10^6
$CD19^+$ B cells	1.1×10^7	1.2×10^4

$CD3^+$ cells/kg, of which $<7.5 \times 10^4$/kg will be $CD45RA^+RO^-$ naïve T cells is feasible. Residual naïve T cells in the infused product could come from the few contaminating T cells in the $CD34^+$ fraction and/or residual naïve T cells in the CD34-negative CD45RA depleted fraction. We have performed six large-scale CD45RA depletions on GCSF-mobilized apheresis products and have achieved a nearly 4 $\log_{10}$ reduction in $CD3^+CD45RA^+$ cells (Table 2). If the T cell dose was adjusted to 5×10^6 $CD3^+$ cells/kg the infused cells would contain $<=2 \times 10^4$ $CD45RA^+CD3^+$ cells/kg after CD45RA depletion, even in the event that only a 2.4 $\log_{10}$ depletion was achieved and the donor had a particularly high proportion of $CD45RA^+$ cells within their original $CD3^+$ population. Thus, the sum of the cell products after CD34 selection and CD45RA depletion is very likely to contain less than $7.5x10^4$ $CD45RA^+CD3^+$ cells/kg. $CD4^+$ T regulatory cells are $CD45RO^+$ and are retained after CD45RA depletion. NK ($CD56^+$) and NK-T ($CD56^+$, $CD3^+$) are present after CD45RA depletion but are reduced by ~1 log, while $CD19^+$ and $CD20^+$ B cells are almost completely removed, which should reduce the risk of EBV-LPD.

3F. <u>T cell responses to pathogens are retained after depletion of $CD45RA^+$cells from G-PBSC</u>

We have evaluated CD45RA depleted hematopoietic cell products for retention of functional memory T cells specific for common pathogens including CMV, EBV, influenza, Candida albicans, and VZV. For persistent viruses such as CMV and EBV, a major fraction of the $CD8^+$ T_M response may reside in the $CD45RA^+$ (T_{EMRA}) subset of T_M, and the use of αCD45RA for depletion of T_N could compromise the transfer of T_M responses to these pathogens. Therefore, we compared the frequency of $CD8^+$ T-cells

specific for known epitopes of CMV and EBV in PBSC before and after depletion of $CD45RA^+$ cells using an interferon gamma Elispot assay. Interferon-producing CMV and EBV-specific $CD8^+$ T-cells were present in both the CD45RA-depleted fraction and unselected PBSC, although there was a reduction in the frequency of these cells in the CD45RA-depleted fraction. However, culture of aliquots of the T-cells before and after CD45RA depletion with CMV, EBV and influenza peptides for 5 days preferentially expanded CMV, EBV, and influenza-specific T_M cells in the CD45RA-depleted fraction, consistent with the enhanced ability of T_{CM} to proliferate and differentiate after restimulation. $CD4^+$ T_M cells are uniformly $CD45RO^+$ $CD45RA^-$ and should be retained in G-PBSC depleted of $CD45RA^+$ T-cells. We analyzed lymphoproliferative responses to CMV, VZV, and Candida antigens before and after depletion of $CD45RA^+$ T-cells from G-PBSC products. To avoid measuring $CD8^+$ T-cells that might respond to soluble proteins that are taken up by APC in the culture and enter the class I pathway, $CD4^+$ T-cells from the CD45RA depleted product were enriched by negative selection prior to plating in the assay. Autologous $CD14^+$ monocytes enriched by immunomagnetic selection were used as APCs. $CD4^+$ T_M responses to all the antigen preparations were detected in the CD45RA depleted product. These results demonstrate that $CD8^+$ and $CD4^+$ memory T cells to common opportunistic pathogens are retained after depletion of $CD45RA^+$ cells. It is also anticipated that pathogen specific memory T cell populations that are transferred with the stem cell graft will expand in the lymphopenic environment post HCT, particularly if CD45RA depletion is effective in preventing GVHD and diminishes the need for post transplant corticosteroid therapy.

Naïve T cells can cause GVHD, but it is uncertain if their removal from a graft could compromise immune recovery. The common opportunistic infections in recipients of grafts that are depleted of all T cells are consequences of reactivation of latent viruses that are controlled by memory T cells. New T_N are generated in the thymus from precursors within 4-6 months of transplant even following complete TCD in older donors. Thus it is anticipated that the capacity of patients to respond to pathogens that they have not previously encountered will ultimately be preserved. The newly generated T_N are likely to be "tolerant" to peptides derived from patient self- proteins due to thymic deletion and/or peripheral suppression of autoreactive T cells.

3G. Overview of the Study

This is a phase 2 study to evaluate selective depletion of naïve T cells from PBSC grafts for preventing acute GVHD in patients with acute leukemia or MDS undergoing allogeneic HLA-identical HCT from a related donor. We will use a myeloablative preparative regimen consisting of fludarabine, thiotepa, and TBI that has resulted in a high rate of engraftment when employed with complete T cell depletion without ATG. The product that will be administered to each patient will consist of an infusion of purified $CD34^+$ cells/kg of recipient body weight, obtained by Miltenyi CliniMACS selection of G-CSF mobilized cells collected from the donor by apheresis, and an infusion of $CD45RA^-$ cells obtained by depletion of $CD45RA^+$ cells from the flow-through remaining after the $CD34^+$ cell selection. As discussed above, our goal is to administer a $CD34^+$ cell dose of $>2.0x10^6$/kg of recipient body weight and a total T cell dose of 1-10 x 10^6 $CD3^+$ cells/kg, of which $<7.5x10^4$/kg will be $CD45RA^+$ RO^- naïve T cells.

The strategy of depleting naïve T cells from stem cell grafts is effective for preventing GVHD in murine models without any post grafting immunosuppression but has not been evaluated in humans. Thus, for the initial clinical application, we plan to study the safety and efficacy of this approach in successive cohorts of patients that will receive progressively less prolonged pharmacologic immunosuppression with single agent tacrolimus (FK506). The rationale for using single agent tacrolimus as GVHD prophylaxis is based on the following considerations. First, randomized studies comparing tacrolimus combined with methotrexate to cyclosporine combined with methotrexate have suggested tacrolimus is superior to cyclosporine for preventing GVHD [4,27]. Second, tacrolimus monotherapy has been shown to result in enhanced neutrophil engraftment, shortened hospitalization, and nearly equivalent rates of GVHD compared with tacrolimus plus methotrexate [28]. Finally, the administration of standard short methotrexate in addition to tacrolimus may cause severe mucosal toxicity due to the increased TBI dose employed in this study. Also methotrexate is a cell cycle active agent and may interfere with homeostatic and antigen-driven expansion of the memory T cells infused with the stem cell graft.

The first cohort of 35 patients will receive tacrolimus alone as post grafting immunosuppression with a planned tacrolimus taper beginning at day 50 in the absence of acute GVHD. If the rate of grade II-IV GVHD in this first cohort is reduced significantly compared to historical controls, a subsequent group of 25 patients will be enrolled and receive a shorter course of tacrolimus for 30 days followed by a rapid taper. If the rate of acute GVHD is <10% in both the first and second cohorts combined, future protocols may evaluate even shorter or less intense immunosuppressive regimens. The risk of graft failure is not expected to be increased given the published results of HCT with complete TCD following conditioning with Flu, Thiotepa and TBI. However, graft failure will also be a primary safety endpoint in the study and early stopping rules for graft failure will be applied. If effective in preventing acute GVHD, this approach to allogeneic HCT may reduce chronic GVHD and improve reconstitution of T cell immunity to pathogens because of the transfer of memory T cells and the reduced need for intensive post transplant immunosuppression. Thus, secondary endpoints will include evaluation of the incidence of chronic GVHD and analysis of the tempo of reconstitution of both total and pathogen-specific T cells.

It is not known if the risk of relapse in HCT with selective T_N depletion will be increased compared with T replete transplantation. Recent studies of complete TCD in acute leukemia have not reported higher relapse rates than those reported with unmanipulated grafts and selective T_N depletion will not remove lymphocytes such as NK, NKT and T_{CM} with the potential to induce a GVL effect[13]. A secondary objective will be to estimate the risk of relapse in recipients of $CD45RA^+$ T cell depleted grafts.

3H. Protocol modification (April 2014)

An interim analysis of protocol 2222 was performed after the first 30 patients were treated with a median of 592 days follow-up for surviving patients. In summary, an excellent rate of engraftment (100%), a very low (12%) rate of chronic GVHD (a secondary endpoint), low rates of relapse (20%) and non-relapse mortality (12%) (secondary endpoints), very good immune reconstitution and survival (overall survival 80%, disease free survival 68%). In the first 30 patients acute GVHD is not lower than the historical benchmark (primary endpoint). However, compared to the

first 13 naïve T cell-depleted HCT recipients (acute GVHD II-IV 85%), acute GVHD appears to be lower among 17 subsequent patients (14-30) (53%) who received antibiotic prophylaxis directed at gram positive organisms (due to a high rate of streptococcus viridans bacteremia amongst the first 13 patients). The original plan was to enroll 35 patients with naïve T cell depletion and tacrolimus monotherapy and if acute GVHD was reduced in this cohort then to enroll a second cohort of patients without tacrolimus. Although the desired reduction in acute GVHD will not be achieved in the first 35 patients, the chronic GVHD rates are very low, the general outcomes are favorable and it is still possible that acute GVHD will in fact be reduced among patients who receive naïve T cell depletion and penicillin. Up to 13 additional patients will be enrolled beyond the 35 patients in cohort 1, potentially extending the total accrual to 48 patients. Statistical considerations are outlined in section 14C.

4. Objectives

4A. Primary objectives

1. Estimate and compare to an appropriate historical value the probability of grades II - IV acute GVHD in successive cohorts of patients who receive $CD45RA^+$ T cell depleted PBSC and:
   a. tacrolimus for GVHD prophylaxis (day -1 to day 50, then tapered from day 50), [Cohort 1].
   b. short course tacrolimus for GVHD prophylaxis (day -1 to day 30, then tapered from day 30), [Cohort 2].
2. Estimate the probability of graft failure in recipients of $CD45RA^+$ T cell depleted PBSC.

4B. Secondary objectives

1. Evaluate immune reconstitution and pathogen-specific T cell reconstitution in recipients of $CD45RA^+$ T cell depleted PBSC.
2. Estimate the probability of transplant-related mortality by day 100 in recipients of $CD45RA^+$ T cell depleted PBSC.
3. Estimate the probability of relapse in recipients of $CD45RA^+$ T cell depleted PBSC.
4. Estimate the probability and severity of chronic GVHD in recipients of $CD45RA^+$ T cell depleted PBSC.

5. Patient Selection

5A. Inclusions

1. Patients who are considered appropriate candidates for allogeneic hematopoietic stem cell transplantation and have one of the following diagnoses:
   a. Acute lymphocytic leukemia in first or subsequent remission
   b. Acute myeloid leukemia in first or subsequent remission
   c. Acute lymphocytic leukemia in relapse or primary refractory disease with a circulating blast count of no more than $10,000/mm^3$
   d. Acute myeloid leukemia in relapse or primary refractory disease with a circulating blast count of no more than $10,000/mm^3$
   e. Refractory anemia with excess blasts (RAEB-1 and RAEB-2) (if the patient has received previous induction chemotherapy within 60 days)
   f. Chronic myelogenous leukemia with a history of accelerated phase or blast crisis (if the patient has received at least one course of induction chemotherapy)
   g. Other acute leukemia (including but not limited to 'biphenotypic',

'undifferentiated' or 'ambiguous lineage' acute leukemia)

2. Patient age 14 to 55 years
3. HLA-identical related donor capable of donating PBSC

5B. Exclusions

1. Patients < 14 or > 55 years of age
2. Patients who are pregnant or breast-feeding
3. Fertile patients unwilling to use contraception during and for 12 months post transplant
4. Patients undergoing second HCT
5. Patients with CNS involvement refractory to intrathecal chemotherapy and/or standard cranial-spinal radiation
6. Patients on other experimental protocols for prevention of acute GVHD
7. Patients who are HIV+
8. Patients with uncontrolled infections for whom myeloablative HCT is considered contraindicated by the consulting infectious disease physician
9. Patients with organ dysfunction
   a. Renal insufficiency (creatinine >1.5 mg/dl)
   b. Cardiac ejection fraction < 45%
   c. DLCO corrected (DLCOcorrected) < 60%
   d. Liver function abnormality. Patients who have LFTs (including total bilirubin, AST and ALT) twice the upper limit of normal should be evaluated by a GI physician unless there is a clear precipitating factor (such as an azole, methotrexate, bactrim or another drug). If the GI physician considers that HCT on protocol 2222 is contraindicated for that patient the patient will be excluded from the protocol. Patients with Gilbert's syndrome and no other known liver function abnormality and patients with reversible drug-related transaminitis do not necessarily require GI consultation and may be included on the protocol.
10. Patients with a life expectancy <3 months from co-existing disease other than the leukemia or RAEB
11. Patients with significant other medical conditions that would make them unsuitable for transplant
12. Patients with a known hypersensitivity to tacrolimus

6. Donor Selection

6A. Inclusions

1. Genotypic or phenotypic HLA-identical family members
2. Adult donors must have adequate veins for leukapheresis or agree to placement of central venous catheter (femoral, subclavian) for collection of PBSC
3. Pediatric donors must have adequate veins for leukapheresis (central venous catheter is not an option for pediatric donors)

6B. Exclusions

1. **Applicable to male patients only**: female donors who have previously given birth to a male child, or have had a second or third trimester miscarriage or termination of pregnancy
2. Donors who have received transfusions of red or white blood cells or platelets
3. Donors with a CD45 gene mutation with aberrant CD45RA isoform expression

4. Donors <14 years of age
5. Donors who for psychological, physiologic, age related or medical reasons are unable to undergo PBSC harvest
6. Donors who for geographical or social reasons are unable to provide a second donation of PBSC in the event of graft failure, EBV lymphoproliferative disease, or relapse requiring donor lymphocyte infusion
7. Donors who are HIV-1, HIV-2, HTLV-1, HTLV-2 seropositive or with active hepatitis B or hepatitis C virus infection
8. Donors who fail eligibility requirements for donation of cells or tissue per section 21 CFR 1271 for donation of a HCT/P will be excluded unless use of the cells complies with 21 CFR 1271.65(b)(1).
9. Female donors who are pregnant or nursing

7. Evaluation and Counseling of Patient and Donor

Patients will be referred to the Fred Hutchinson Cancer Research Center (FHCRC) or Yale University School of Medicine (YUSM) for consideration of a hematopoietic cell transplant. Both patient and donor will be evaluated separately. The protocol will be discussed thoroughly with patient, donor and other family members if appropriate, and all known risks to the patient and donor will be described. The procedure and alternative forms of therapy will be presented as objectively as possible, and the risks and hazards of the procedure explained to the patient or, in the case of minors, to the patient's responsible legal guardian. Consent at FHCRC and YUSM will be obtained using facility specific forms approved by the FHCRC Institutional Review Board. A summary of the conference detailing what was covered will be dictated for the medical record.

8. Protocol Registration

8A. Overall Procedures:

Patients enrolled at FHCRC will be assigned to the protocol by the SCCA (Seattle Cancer Care Alliance) Clinical Coordinator. Once full consent has been obtained the SCCA Consent Coordinator will register the patient with the Registration Office (206) 667-4728, between 8:30 am and 4:00 pm, Monday through Friday. After hours, the Registration Office can be reached by paging (206) 995-7437.

Allocation of a study number (unique patient identifier) is required for patients enrolled at each site. Each site will keep a log of patients enrolled.

Once a study number is assigned patients will then be entered into the P2222 (**Protocol Patient Performance Program** (PPPP) database by FHCRC study staff.

8B. YUSM Procedures:

When a patient at YUSM is thought to be eligible, pre-screening consent will be obtained from the donor and full consent will be obtained from the recipient. When signed consents are obtained, YUSM study staff will notify the FHCRC study research nurse by email and provide copies of the signed consent forms (consent for patient and pre-screening consent for the donor) and patient and donor registration forms via fax (206) 667-6056 or email.

The FHCRC study nurse will coordinate with the FHCRC regulatory coordinator to ensure that the completed patient and donor registration forms and consent documents are

faxed to the FHCRC Registration Office. The FHCRC Registration Office will generate a unique patient identifier.

The unique patient identifier will be provided by email to the designated YUSM research nurse within 48 business hours after receipt of the signed patient consent and donor pre-screening consent forms.

If pre-screening indicates that the donor is eligible, which includes primarily documentation of normal CD45RA expression, a signed donor consent form for continued participation in the study will be obtained and provided via fax to FHCRC (206) 667-6056 and the FHCRC research staff will fax the donor consent to the FHCRC Registration Office. The source documents for pre-screening testing of CD45RA expression will be submitted to the FHCRC research nurse when available or may be held until the full eligibility package is assembled for both patient and donor.

The FHCRC study nurse and FHCRC PI will review all source documents supporting eligibility criteria for the patient and donor and will verify if the patient and donor are eligible.

An email will be sent to Dr. Warren Shlomchik (warren.shlomchik@yale.edu) containing the FHCRC PI's determination of eligibility within 48 hours (during the Monday-Friday workweek) of receiving all required source documentation.

Informed consent must be signed by the transplant recipient or his/her legal guardian and confirmation of eligibility must be verified by the coordinating center PI prior to the performance of any study-specific treatment.

Plan of Treatment

9A. Conditioning Regimen

The conditioning regimen will consist of fludarabine, thiotepa and fractionated total body irradiation (TBI) and will be administered as outlined below and shown schematically in Table 3. Note that for chemotherapy administered during conditioning and other drugs listed in this protocol, in keeping with institutional practice it is acceptable to administer a dose within 10% of the protocol specified dose.

1. TBI will be given as 165 cGy fractions twice per day x 4 days – total dose 1320cGy (days -10 to -7).
2. After completing the TBI, patients will be treated with thiotepa 5 mg/kg/day (adjusted body weight –see appendix I) administered intravenously over approximately 4 hours on each of two consecutive days (days -6 and -5). If actual weight is *less* than ideal body weight, actual body weight will be used. The total dose is 10 mg/kg (adjusted body weight - see appendix I).
3. Patients will receive fludarabine 25 mg/m^2/day (m^2 always to be based on actual weight see appendix I) administered intravenously over approximately 30 minutes for 5 days beginning on the first day of thiotepa (days -6 to -2).
4. Day -1 will be a day of rest.
5. GCSF-mobilized CD34 enriched PBSC and CD45RA depleted cells will be infused on day 0.

Table 3. Conditioning regimen

Day relative to infusion of GCSF mobilized CD34 enriched PBSC and CD45RA depleted cells

Treatment	-10	-9	-8	-7	-6	-5	-4	-3	-2	-1	0	1
TBI 165 cGy BID x 4 days	X	X	X	X								
Thiotepa (5 mg/kg/day x 2 days)					X	X						
Fludarabine (25 mg/m^2/day x5 days)					X	X	X	X	X			
Infusion of G-PBSC CD34$^+$ and CD45RA$^-$ cells											X	+/-

9B. Central Nervous System Prophylaxis and Testicular Irradiation

Patients will have a diagnostic lumbar puncture prior to the preparative regimen. Intrathecal chemotherapy will be given according to institutional standard practice if a. the CSF is positive for malignant cells; b. the patient has a prior history of CNS disease; c. the patient has a history of ALL; or d. the patient is otherwise deemed to be at significant risk of CNS disease. No dose of intrathecal therapy will be given within 72 hours of CD34 enriched PBSC infusion. Male patients with ALL will receive a 400 cGy testicular irradiation boost pre-transplant during the conditioning regimen.

9C. Collection of G-CSF mobilized PBSC

1. G-CSF administration to donors: All donors will receive G-CSF 16 μg/kg/day administered as 8 μg/kg two times per day by subcutaneous injection for 5 consecutive days on day -5 thru day-1, and in some cases in the morning on Day 0. Donors who fail to mobilize adequately and require a day +1 collection may also receive GCSF 8 μg/kg in the pm on Day 0. G-CSF doses will be administered at approximately 8:00 a.m. and between approximately 4:30 pm and 8 pm each day in the Outpatient Department. For Yale patients, the weekend p.m. dose may be administered in the in-patient BMT unit. The schedule of G-CSF administration and PBSC collections will be determined when the schedule for the conditioning regimen and day 0 is established and must be confirmed with the personnel in the Apheresis Facility. Day 0 should be fixed on a Tuesday-Thursday.

2. PBSC collection by apheresis: Donors may undergo vein to vein apheresis collections. If apheresis cannot be performed by this technique, a percutaneous Mahurkar catheter may be inserted for adult donors. PBSCs will be collected using standard apheresis procedures. We will aim to process up to 18L liters of whole blood per collection for all donors. The first apheresis will be performed on day -1, and stored in the refrigerator at 4° C overnight according to the Standard Operating Procedures for overnight storage of PBSC products at each institution. A second PBSC collection will be performed on the morning of day 0. Prior to cell selection, a sample of the PBSC products will be analyzed for the content of total nucleated cells, CD34$^+$ cells, CD3$^+$, CD3$^+$ CD45RA$^+$ RO$^-$ and total CD45RA$^+$ cells and an aliquot of cells will be obtained for subsequent analysis of other cell subsets (see section 9D.3 below). Cell selection will be performed on day 0 (9D & Appendix A) and the final product will be tested for quality control (9D.3) and infused on day 0 (9E). In a small minority of cases CD34$^+$ cell target numbers may not be achieved by day 0 and a third PBSC collection will be required on day 1. This PBSC product will not undergo any cell selection process and will be infused on day 1 (9D and 9E).

Table 4. Treatment schema-donor

Day relative to infusion of GCSF mobilized CD34 enriched PBSC and CD45RA depleted cells

Treatment	-5	-4	-3	-2	-1	0	1
G-CSF 8 μg/kg s.c. b.i.d.	X X	X X	X X	X X	X X	+/- +/-	
Apheresis					X	X	+/-

9D. PBSC graft engineering - $CD34^+$ cell selection and depletion of $CD45RA^+$ cells

- We will perform cell selections only when the PBSC product from days -1 and 0 combined contains $\geq 5x10^6$ $CD34^+$ cells/kg of recipient body weight to ensure that the $CD34^+$ cell dose will be $\geq 2x10^6$ $CD34^+$ cells/kg of recipient body weight.
- If the PBSC product from days -1 and 0 combined contains $<5x10^6$ $CD34^+$ cells/kg of recipient body weight the PBSCs will be infused without any cell selection and the patients will not be included in the analysis of study efficacy end points. These patients will receive GVHD prophylaxis with tacrolimus and methotrexate (see section 9F). Up to 10% of patients enrolled on protocol 2222 may receive a G-PBSC product that does not undergo any cell selection.
- For the patients in whom cell selection is performed we expect to achieve:
  - The CD34 goal of a minimum of 2.0×10^6 $CD34^+$ cells/kg of recipient body weight in all patients
  - A maximum of 7.5×10^4 $CD45RA^+RO^-CD3^+$ T cells/kg recipient body weight and a range of $1\text{-}10 \times 10^6$/kg total $CD3^+$ T cells in >90% of patients.

The cell selection will be performed as outlined below and in the flow chart provided as Appendix A.

1. $CD34^+$ cell selection (Also see Appendix A)
   The apheresis products obtained on day –1 and day 0 will be processed by $CD34^+$ cell selection using the clinical-grade Miltenyi anti $CD34^+$ conjugated iron dextran microbeads and magnetic selection with the CliniMACs device using the institution's Standard Operating Procedure. The goal is to obtain a target CD34 cell dose of $>2.0x10^6$/kg of recipient body weight to administer with the engineered graft and this goal is expected to be achieved for all products that undergo cell selection. The CliniMACS system of CD34 selection results in a 4-5 $\log_{10}$ depletion of $CD3^+$ cells which will result in a median CD3 cell dose of $\leq 6x10^4$/kg of which 30 –70% may be $CD45RA^+RO^-$.
2. $CD45RA^+$ cell depletion to remove T_N cells (See also Appendix A)
   The CD34-depleted flow through fraction(s) will be collected and processed to deplete $CD45RA^+$ cells using clinical grade anti-CD45RA-conjugated iron dextran beads and magnetic selection with the CliniMACS device according to the institution's standard operating procedures. The phenotype and content of T cells that remain in the product after removal of $CD45RA^+$ cells will be determined by flow cytometry (see section 9D3, below). The goal is to administer $<7.5 \times 10^4$/kg of $CD45RA^+RO^-$ cells and a total $CD3^+$ T cell dose of $1\text{-}10 \times 10^6$/kg to the patient including both the $CD34^+$ enriched fraction and the CD45RA depleted fraction. To

achieve these total T cell numbers, a proportion of the CD45RA depleted cells will be administered to the patient following administration of the positively selected $CD34^+$ cell product.

3. Analysis of the engineered cell products Samples will be taken from the apheresis products before and after the CD34 selection and depletion of $CD45RA^+$ cells, and analyzed as follows (also see Appendix B "Product Testing"):
   a. Viability testing
   b. Sterility testing
   c. Total nucleated cell count
   d. Immunophenotyping by flow cytometry will be performed on the initial apheresis product and cells that have completed the selection process:
      i. The following cell subsets will be enumerated to guide cell selection and to determine whether the goals for the composition of the product are achieved:
         - $CD34^+$ cells
         - $CD3^+$ cells
         - $CD3^+$ $CD45RA^+$ RO^- cells
      ii. Additional cell markers will be evaluated and may include
         - $CD3^+$ $CD45RA^+$ $CD45RO^-$ $CCR7^+$ (T_N)
         - $CD8^+CD45RA^+$ $CD45RO^-$ $CCR7^+$ (CD8 T_N) and $CD4^+CD45RA^+$ $CD45RO^-$ $CCR7^+$ (CD4 T_N)
         - $CD3^+$ $CD45RO^+$ (T_M), $CD3^+CD8^+$ $CD45RO^+$ (CD8 T_M) and $CD3^+$ $CD4^+$ $CD45RO^+$ (CD4 T_M)
         - $CD3^+$ $CD45RO^+$ $CCR\text{-}7^+$ (T_{CM}) and $CD3^+$ $CD45RO^+$ $CCR\text{-}7^-$ (T_{EM})
         - $CD8^+$ $CD45RO^+$ $CCR\text{-}7^+$ (CD8 T_{CM}) and $CD8^+$ $CD45RO^+$ $CCR\text{-}7^-$ (CD8 T_{EM}), and $CD4^+$ $CD45RO^+$ $CCR\text{-}7^+$ (CD4 T_{CM}) and $CD4^+$ $CD45RO^+$ $CCR\text{-}7^-$ (CD4 T_{EM})
         - $CD3^{+/-}$ $CD56^+$, $CD3^{+/-}$ $CD16^+$, $CD14^+$, $CD19^+$ and $CD20^+$
         - $CD4^+$ $CD25^+FoxP3^+$.

a. Specific T cell responses to pathogen-derived antigens may be performed on aliquots of the CD45RA depleted cell product and PBSC prior to CD45RA depletion using functional assays which may include ELISPOT, intracellular cytokine staining and/or tetramer analysis.

9E. PBSC Infusion

The $CD34^+$ selected and CD45RA-depleted products will be infused through a central venous catheter on day 0 of the transplant. The $CD34^+$ product will be infused first, followed as soon as possible thereafter, by the CD45RA-depleted product (see Appendix D). If additional $CD34^+$ cells are required (see section 9D.1 and Appendix A and D) unselected PBSC will be administered through the central venous catheter on day 0 or 1.

9F. Post-transplant immunosuppression

In the first cohort of 35 patients, GVHD prophylaxis will consist of tacrolimus (FK506) alone for 50 days followed by a standard taper if there is no GVHD (see section 9F.1b). If in the first cohort, the rate of acute GVHD grades II or higher is significantly lower than historical controls (see section 14C Statistical Analysis) and the rate of acute GVHD grades III-IV does not exceed 15%, a subsequent cohort of 25 patients will receive a shorter course of tacrolimus alone for 30 days post transplant followed by a more rapid taper if they do not develop GVHD (see section 9F.1c).

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In the event that the target cell doses cannot be achieved for a patient using the cell selection procedure described in section 9D.1 and 2 and Appendix A and the patient requires the infusion of unmanipulated G-PBSC methotrexate will be administered in addition to tacrolimus as immunosuppression.

1. Tacrolimus administration
   a. Tacrolimus will be administered beginning on day -1 at a dose of 0.03 mg/kg/day by continuous IV infusion per institutional standard practice guidelines. Tacrolimus doses are based on actual body weight. If actual weight is *more* than ideal body weight, it is recommended to use adjusted body weight. Conversion to the oral formulation of tacrolimus (IV: PO ratio of 1:4) may be made when oral feeding is established. Oral tacrolimus is recommended to be given in two divided daily doses every 12 hours on an empty stomach.
   b. For the first cohort of 35 patients, if there is no evidence of grade II-IV acute GVHD on or prior to day 50, tacrolimus should then be tapered at the rate of approximately 5% of the day 50 dose each week for liquid, and 20% of the day 50 dose per month for capsules.
   c. In the second cohort of 25 patients if there is no evidence of grade II GVHD on or prior to day 30, tacrolimus should then be tapered at the rate of approximately 8% of the day 30 dose each week for liquid, and 33% of the day 30 dose per month for capsules.
   d. If there is evidence of acute GVHD, then the standard recommendations for treatment of acute GVHD and tapering of immunosuppression should be followed according to institutional practices. Patients with GVHD may be enrolled in investigational protocols for GVHD treatment.
   e. If there is evidence of disease progression and no evidence of GVHD prior to day 28, patients in either cohort should taper tacrolimus and all other immunosuppressive agents within 2 weeks and be observed for the development of GVHD. The taper may be accelerated or tacrolimus may be discontinued, as clinically indicated.

2. Monitoring of tacrolimus levels and dose adjustment
   a. Tacrolimus levels should be maintained in the range of 5-15 ng/ml. Whole blood trough levels should be obtained on approximately day 2, then approximately once each week or more often if clinically indicated, such as when IV to PO dosing is initiated. Dose adjustments are made if the levels are outside the therapeutic range, or if there is evidence of toxicity that may be related to tacrolimus.
   b. When initiating therapy with voriconazole in patients receiving tacrolimus, it is recommended that the tacrolimus dose be reduced to one-third of the original dose, (a 67% reduction) and followed with frequent monitoring of the tacrolimus blood levels. When voriconazole is discontinued, tacrolimus levels should be carefully monitored and the dose increased as necessary.
   c. Weekly tacrolimus levels can be discontinued during a tacrolimus taper when the dose has been reduced by 25% if the patient has an adequate oral intake, volume status, renal function and the absence of toxicities that might be attributed to tacrolimus.
   d. Blood pressure, renal function tests (creatinine, BUN), electrolytes and magnesium should be monitored regularly as per institutional standard practice

guidelines.

3. Methotrexate administration

Most patients treated on this protocol will not receive any methotrexate. If patients do require the infusion of unmanipulated G-PBSC because of inadequate $CD34^+$ cell content before or after cell processing they will receive methotrexate in addition to tacrolimus as immunosuppression. Methotrexate should be administered to this subgroup of patients on days +1, +3, +6 and +11 at a dose of 5 mg/m^2 by IV push. Calculation of m^2 should be according to institutional standard practice guidelines. The first dose of methotrexate should be given approximately 24 hours after completion of all cell infusions, and no sooner than 24 hours after completion of cell infusions. If clinically necessary methotrexate levels may be obtained and/or methotrexate dose adjusted according to institutional standard practice guidelines.

9.G. Use of hematopoietic growth factors

There will not be routine post-transplant use of growth factors in patients enrolled on this protocol. Growth factors may be recommended by the Attending Physician to manage slow engraftment as indicated in Section 12 E.

10. Evaluation

10A. Donor evaluation

Pre-donation evaluation should be conducted as per standard institutional practice. The following should be obtained for all donors:

1. Female donors should have a birth history obtained. Male patients with female donors who are pregnant, have given birth to male children or who have had a second or third trimester miscarriage (or termination of pregnancy) are not eligible for inclusion in this protocol as they may have been immunized to minor H antigens.
2. All donors should have a blood transfusion history obtained. Donors who have received transfusions of red or white blood cells or platelets are not eligible for inclusion in this protocol as they may have been immunized to minor H antigens.
3. A full vaccination history.
4. Testing by flow cytometry for the CD45 gene mutation with aberrant CD45RA isoform expression should have been completed prior to enrollment on protocol 2222. Donors with aberrant CD45RA isoform expression are not eligible for inclusion on this protocol.
5. Confirmatory HLA typing between recipient and donor.
6. Leukocytotoxic and/or Fluorescence Activated Cell Sorter cross match between recipient and donor. For Yale patients, One Lambda Luminex mixed screen kit as well as single antigen kit will be used for HLA antibody screening, and anti-human globulin enhanced complement-dependent lymphocytotoxicity method will be employed for crossmatching.
7. ABO and Rh typing and two way red cell cross match between recipient and donor.
8. DNA for storage for chimerism studies. For FHCRC patients, samples will be sent to the Clinical Immunogenetics Laboratory (206) 288-1128. For Yale patients, samples will be sent to the Molecular Diagnostics Laboratory (203) 688-6985.
9. Screening for high-risk behavior and HepBsAg, antiHepB core antibody, HBV NAT, antiHep C antibody, HCV NAT, HTLV-1 and HTLV-2 antibodies, a serologic test for syphilis, and HIV (1 & 2) antibodies and HIV NAT, all performed within 30 days of donation and West Nile Virus NAT testing and Trypanosoma antibody testing

according to institutional standard practice. Tests are performed using FDA licensed, cleared, and approved test kits in a CLIA-certified laboratory.

10. CMV and EBV serologies performed within 30 days of donation.
11. Serum pregnancy qualitative within 2 weeks of the start of conditioning
12. G-CSF and monitoring blood draws (including CBC and Hepatic Function Panel with LDH) should be conducted per institutional standard practice guidelines.
13. If collection of donor lymphocytes is required post transplant for the treatment of relapse or EBV post transplant lymphoproliferative disorder, the PBSC donor should be re-evaluated as per institutional standard practice.
14. Research Tests: A 90 cc sample of donor blood should be collected (preferably before the donor starts GCSF) in heparinized or ACD tubes for immunologic studies, specifically T_M responses to pathogens by functional assays which may include ELISPOT, lymphoproliferation assays, intracellular cytokine staining and/or tetramer analysis. SCCA patients send the sample to the Riddell lab D3-313 and contact the protocol 2222 research technician at (206) 667-4804 or Marie Bleakley at (206) 469-4487. For Yale patientssend the sample to the FHCRC Riddell lab. Some assays on these cells may also be performed at Yale.

10B. Patient pre-transplant evaluation

Patient pre-transplant evaluation should be conducted as per standard institutional practice. The following should be obtained for all patients:

1. History
   a. Possible antecedent causes for the development of leukemia or MDS including prior cytotoxic therapy.
   b. Hematologic, cytogenetic and flow cytometric findings at diagnosis and at the time of enrollment.
   c. Prior therapies and response to therapy

2. Laboratory evaluation
   a. Bone marrow aspirate for morphology with standard cytogenetics and where appropriate, molecular cytogenetics, within 30 days of the start of conditioning.
   b. Bone marrow flow cytometry for determination of blast counts within 30 days of the start of conditioning.
   c. Lumbar puncture with CSF evaluation within 30 days of the start of conditioning.
   d. Confirmatory HLA typing between recipient and donor.
   e. Leukocytotoxic and/or Fluorescence Activated Cell Sorter cross match between recipient and donor. For Yale patients, One Lambda Luminex mixed screen kit as well as single antigen kit will be used for HLA antibody screening, and anti-human globulin enhanced complement-dependent lymphocytotoxicity method will be employed for crossmatching.
   f. ABO and Rh typing and two way red cell cross match between recipient and donor.
   g. DNA for storage for chimerism studies. For FHCRC patients, samples will be sent to the Clinical Immunogenetics Laboratory (206) 288-1128. For Yale patients, samples will be sent to the Molecular Diagnostics Laboratory (203) 688-6985.
   h. HepBsAg, antiHepB core antibody, antiHep C antibody, HTLV-1, and HTLV-2 antibodies, a serologic test for syphilis, and HIV (1 & 2) and HIV p24 antigen or HIV PCR within 30 days of the start of conditioning.
   i. CMV, EBV, HSV, VZV serologies within 30 days of the start of conditioning.

j. CMV and EBV plasma PCR and aspergillus galactomannan EIA obtained and run within 2 weeks prior to starting conditioning.
k. Quantitative immunoglobulins should be obtained within 30 days of the start of conditioning.
l. CBC within 2 weeks of the start of conditioning.
m. LFTs, to include ALT, AST, and Bili T/D, within 2 weeks of the start of conditioning.
n. Serum chemistry, to include Na, K, Cl, CO_2, BUN, Cr, Ca, Mg, and Phos, with in 2 weeks of the start of conditioning.
o. Serum pregnancy qualitative within 2 weeks of the start of conditioning.
p. Research Tests: For FHCRC patients only: Approximately 90 cc of peripheral blood, in heparinized or ACD tubes should be sent to the FHCRC Riddell lab for immunological studies.

3. Other evaluations:
   a. MUGA scan or echocardiogram within one month of the start of conditioning.

10C. Patient post transplant evaluation guidelines

See Institutional standard practice Policy Guidelines for standard evaluation procedures during the first 100 days post transplant, evaluation prior to departure and long-term follow-up. In addition:

1. Bone marrow aspiration should be performed at baseline and on approximately days +28, day +56 and between days +80 and + 100. Bone marrow aspiration may also be performed at approximately one year after transplant. Additional bone marrow aspirations and/or biopsies may be performed at the discretion of the treating physicians.
2. Donor and recipient chimerism of $CD3^+$ and $CD33^+$ subsets in the peripheral blood should be evaluated at approximately day +28 +56, and +80 by STR polymorphism. Peripheral blood chimerism studies may also be performed at approximately 6 and 12 months post transplant.
3. Quantitative immunoglobulins should be assessed at baseline and on approximately days +30, +60 and + 90 and if possible on approximately day +180 and +360. Antibody responses to recall antigens (tetanus toxoid, diphtheria toxoid and measles virus) should be assessed at one year post transplant if possible.
4. CMV PCR should be performed once every week until day 100 or departure, and after departure according to institutional guidelines. Preemptive antiviral therapy should be instituted for a positive PCR according to institutional guidelines.
5. Surveillance for EBV should be performed using a quantitative EBV DNA PCR assay approximately weekly until day 100 or departure. Infectious disease consultation should be obtained for patients who develop EBV DNA levels of >1000 copies/ml plasma on any test. For Yale patients, EBV DNA is reported as number of genomes/100,000 WBC's. Infectious disease consultation will be obtained if EBV DNA levels are > 1000 genomes/100,000 WBC's.
6. Galactomannan EIA (GMEIA) should be performed approximately once per week between day 28 and day 100 or departure. Infectious disease consultation should be obtained for patients who develop a positive GMEIA.
7. Research Tests:
   a. Immune reconstitution should be assessed on approximately day +28, +56, and between approximately day +80 and +100. Where possible immune reconstitution should also be assessed at approximately day +180 and +360. SCCA patients send

approximately 90 cc of heparinized (or ACD) peripheral blood to the Riddell lab D3-313 contact the protocol 2222 research technician on (206) 667-4804 or Marie Bleakley (206) 469-4487. For Yale patients, 90 cc of heparinized (or ACD) peripheral blood will be sent to the FHCRC Riddell lab.

i. Lymphocyte subsets will be enumerated using flow cytometry. Cell markers may include:
   - $CD3^+$ $CD45RA^+$ $CD45RO^-$ $CCR7^+$ (T_N) cells
   - $CD8^+CD45RA^+$ $CD45RO^-$ $CCR7^+$ (CD8 T_N) and $CD4^+CD45RA^+$ $CD45RO^-$ $CCR7^+$ (CD4 T_N) cells
   - $CD3^+$ $CD45RO^+$ (T_M), $CD3^+CD8^+$ $CD45RO^+$ (CD8 T_M) and $CD3^+$ $CD4^+$ $CD45RO^+$ (CD4 T_M) cells
   - $CD3^+$ $CD45RO^+$ $CCR\text{-}7^+$ (T_{CM}) and $CD3^+$ $CD45RO^+$ $CCR\text{-}7^-$ (T_{EM}) cells
   - $CD8^+$ $CD45RO^+$ $CCR\text{-}7^+$ (CD8 T_{CM}) and $CD8^+$ $CD45RO^+$ $CCR\text{-}7^-$ (CD8 T_{EM}), cell number and $CD4^+$ $CD45RO^+$ $CCR\text{-}7^+$ (CD4 T_{CM}) and $CD4^+$ $CD45RO^+$ $CCR\text{-}7^-$ (CD4 T_{EM}) cells.
   - $CD3^{+/-}$ $CD56^+$, $CD3^{+/-}$ $CD16^+$, $CD14^+$, $CD19^+$ and $CD20^+$ cells
   - $CD4^+$ $CD25^+FoxP3^+$ cells.

ii. Specific T cell responses to pathogens (such as CMV, EBV, Candida, and VZV) will be performed using functional assays which may include ELISPOT, lymphoproliferation assays, intracellular cytokine staining, and/or tetramer analysis.

iii. Specific T cell responses to leukemia associated antigens may be performed in a subset of patients using functional assays including intracellular cytokine staining and/or tetramer analysis.

iv. T cell receptor excision circle (TREC) analysis may be performed on a subset of patients.

2222.00

A summary of patient evaluations post-transplant is provided in Table 5.

Table 5. Patient evaluations over the course of the study

	Approximate days post transplant															
Study Assessments/ Testing	Baseline	0	7	14	21	28	35	42	49	56	63	70	77	Between 80-100	180	360
History, physical exam, height[1] and weight	X					X				X				X		X
Karnofsky performance status	X													X		X
Automated CBC with differential, platelet count	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
Serum chemistry [2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X
CMV PCR [3]	X		X	X	X	X	X	X	X	X	X	X	X	X		
EBV PCR	X		X	X	X	X	X	X	X	X	X	X	X	X		
Aspergillus GM-EIA	X					X	X	X	X	X	X	X	X	X		
Quantitative Immunoglobulins for IgG, IgA and IgM	X					X				X				X	X	X
Quantitative blood lymphocyte subset evaluation						X				X				X	X	X
Antibody response to recall antigens																X
Peripheral blood T cell & myeloid chimerism ($CD3^-$ & $CD33^+$)	X Baseline DNA					X				X				X	X	X
Bone marrow aspirate/biopsy[4]	X					X				X				X		X
Morbidity assessments[5]			X	X	X	X	X	X	X	X	X	X	X	X	X	X
Acute GVHD			X	X	X	X	X	X	X	X	X	X	X	X		
Chronic GVHD[6]														X		X
Skin biopsy														X		
Oral medicine consult														X		
Pulmonary function test including DLCO	X													X		X
Research Tests																
Blood lymphocyte flow cytometry analysis and specific T cell responses	X (FHCRC patients only)					X				X				X	X	X

1. Height on baseline only
2. Serum Chemistry should include the following: Na, K, Cl, CO2, BUN, Cr, Ca, Mg, Phos, ALT, AST, and BILIT/D
3. CMV PCR or antigen monitoring is suggested to continue on a weekly basis for at least one year beyond day 100 for patients who have had CMV reactivation in the first 100 days and/or have active GVHD requiring steroids or other agents.
4. Bone marrow aspiration; evaluation with **a.** histopathology **b.** flow cytometry **c.** cytogenetics (FISH and PCR informative for disease status eg BCR-ABL for Ph+ ALL)
5. Morbidity assessments refer to evaluation for adverse events including standard clinical and laboratory evaluation.
6. Chronic GVHD evaluation should be performed between approximately day 80 and 100, and on day 360 where possible. Patients with symptoms or signs compatible with chronic GVHD between day 100 and day 360, based on report by the PMD and/or patient, should be asked to return for a LTFU chronic GVHD consult.

Post transplant time points represent guidelines for performance of required evaluations. Due to numerous factors influencing scheduling (pt and provider availability, testing services limitations etc), variation in evaluation performance dates is anticipated and acceptable to the protocol (e.g., within +/- 7 days of time points < day 100; +/- 30 days for time points > day 100).

11. Drugs, Irradiation and PBSC Administration - Toxicities and Complications.

Note that for chemotherapy administered during conditioning and other drugs listed in this protocol, in keeping with institutional practice it is acceptable to administer a dose within 10% of the protocol specified dose.

11A. Total Body Irradiation (TBI)

1. TBI will be given as 165 cGy fractions twice per day x 4 days (days -10 to -7) to all patients using the linear accelerator at a rate of 6-7 cGy/min. Dosimetry calculations will be performed by the radiation oncologist.
2. Toxicity:
   a. Myelosuppression is the major dose limiting toxicity.
   b. Erythema may occur in the first 24 hours.
   c. Hyperpigmentation may occur in the first month following TBI.
   d. Oral ulceration, anorexia, nausea, vomiting and diarrhea, fatigue and alopecia occur frequently.
   e. Parotitis
   f. Decreased production of saliva and tears
   g. Hepatic dysfunction and rarely liver failure.
   h. Late effects include cataracts, growth failure, gonadal failure and sterility, hypothyroidism, pulmonary dysfunction and secondary malignancies.

11B. Thiotepa

1. Dosage: Thiotepa will be administered in a dose of 5 mg/kg/day (adjusted body weight) IV over approximately 4 hours for 2 consecutive days (day -6 and day -5). The total dose is 10 mg/kg (adjusted body weight). If actual weight is *less* than ideal body weight, actual body weight will be used. Thiotepa is available in 15 mg vials and is reconstituted with sterile water resulting in an isotonic solution with 10 mg/ml of thiotepa.
2. Toxicity:
   a. The major dose limiting toxicity of thiotepa is myelosuppression.
   b. Oral ulceration, anorexia, nausea, vomiting and diarrhea, fatigue and alopecia occur frequently.
   c. Occasionally patients develop a skin rash that involves darkening of the skin and peeling, particularly in the axillary and inguinal folds. Thiotepa is secreted in sweat, therefore the axillary and inguinal areas should be washed twice daily during administration and for 2 days after administration.
   d. Dizziness and headache
   e. Hepatic toxicity including elevation in bilirubin and transaminases, and hepatic damage can occur.
   f. Rarely reported toxicities include CNS toxicity with somnolence, confusion, seizures, forgetfulness and inappropriate behavior.
   g. Allergic reactions during infusion occur rarely.
   h. Late effects include sterility and secondary malignancies.

11C. Fludarabine

1. Dosage: Fludarabine will be administered in a dose of 25 mg/m^2/day (m^2 always based on actual body weight) IV over approximately 30 minutes for 5 consecutive days (day -6 to -2). The total dose of fludarabine will be 125 mg/m^2.
2. Toxicity:
   a. Immunosuppression is the major toxicity of fludarabine.
   b. Oral ulceration, anorexia, nausea, vomiting and diarrhea, fatigue and alopecia occur frequently.
   c. Myelosuppression (lymphopenia, granulocytopenia, and anemia) is common.
   d. Numbness and tingling in hands or feet and visual changes occur and rarely

somnolence, mental state changes, cortical blindness, coma and other neurotoxicity

e. Other reported toxicities include rash, hepatocellular toxicity, hemolytic anemia and interstitial pneumonitis.

11D. PBSC infusion

Refer to Appendix D for infusion of selected cells.

11E. G-CSF

For donor stem cell mobilization- see section 9C.

11F. Tacrolimus administration

1. Also see section 9F for information about tacrolimus administration and dosage adjustments.
2. Administration and dosage:
   a. Intravenous dosing - The IV formulation of tacrolimus is supplied as a sterile solution of 5 mg/ml ampules in polyoxyethylated castor oil (Cremophor FCL). The drug is diluted in D5W in glass or other non-PVC container. The standard mode of IV administration is by continuous infusion over 22-24 hours. Tacrolimus should be initiated as an IV continuous infusion on day –1 at a dose of 0.03 mg/kg/day based on actual body weight. If actual weight is greater than ideal body weight, it is recommended to use adjusted body weight.
   b. Oral dosing – The oral formulation of tacrolimus is supplied as 0.5 mg, 1 mg or 5 mg capsules or as oral syrup (0.5 mg/ml). For better absorption, it is recommended that Tacrolimus capsules be taken on an empty stomach. Tacrolimus should not be taken with grapefruit juice or other beverages containing bergamottin as it may increase blood levels. If patient vomits within one hour of oral administration, repeat dosing is recommended and if vomiting persists, switch to IV administration is recommended.
   c. Conversion from IV to PO dosing of Tacrolimus. Patients should be converted to an oral dose at 4 times the IV dose to be given in divided (Q 12 hour) doses.
3. Toxicity

   Side effects are generally reversible and may include:
   a. Rise in serum creatinine, electrolyte wasting, hemolytic uremic syndrome, and renal failure.
   b. Nausea and vomiting and hepatic dysfunction.
   c. Hypertension,
   d. Increases in cholesterol and triglycerides
   e. Paresthesia, tremors, seizures, headache, insomnia, dizziness, depression, confusion, hallucinations, psychosis, myoclonus, neuropathy, agitation.
   f. Blurred vision, photophobia.
   g. Hirsutism

12. Protocol Enrollment and Special Considerations

12A.

Projected Target Accrual
ETHNIC AND GENDER DISTRIBUTION CHART

TARGETED / PLANNED ENROLLMENT: Number of Subjects			
Ethnic Category	Sex / Gender		
	Females	**Males**	**Total**
Hispanic or Latino	1	1	2
Not Hispanic or Latino	24	34	58
Ethnic Category Total of All Subjects*	25	35	60
Racial Categories			
American Indian / Alaska Native	0	0	0
Asian	0	1	1
Native Hawaiian or Other Pacific Islander	0	0	0
Black or African American	1	1	2
White	24	33	57
Racial Categories: Total of All Subjects*	25	35	60

12B. Infection Prophylaxis

1) Antibiotics

(a) Prophylactic antibacterial antibiotics should be used for all patients that develop neutropenia (ANC <500/ml) according to institutional standard practice guidelines or according to currently active protocols.

(b) In order to prevent early *Streptococcus viridians* bacteremia all patients should receive additional prophylactic therapy according to institutional practice. The recommended standard regimens are:

(i) ***FHCRC Standard:*** Penicillin VK 1 gram PO BID starting at day -2 until not able to take oral therapy and then IV Penicillin G one million units IV every six hours (4 million units total) until day +10.

(ii) ***Yale Standard:*** Ceftriaxone 1gm IV Q24 beginning day -2.

(iii) ***Penicillin Allergic***: IV Vancomycin 15 mg/kg IV every 12 hours from day -2 through day +10.

(c) Neutropenic fever: In patients with any active mucositis and neutropenia, IV Vancomycin (15 mg/kg IV at least every 12 hours) or an alternative antibiotic regimen covering streptococcus viridans should be given in addition to the other empiric neutropenic fever antibiotic therapy specified by institutional standard

practice guidelines (e.g. Cefazidime). Prophylactic penicillin or ceftriaxone may be discontinued when vancomycin or an alternative anti-streptococcus viridans agent is initiated.

2) Prophylactic fluconazole (400 mg/day) should be used from the first day of conditioning or sooner until day +75 post transplant unless patients are participating in a study of antifungal prophylaxis.
3) Preemptive therapy for CMV reactivation should be administered according to institutional standard practice guidelines.
4) Infectious disease consultation should be obtained for patients who develop EBV DNA levels of >1000 copies/ml plasma on any test. For Yale patients, EBV DNA is reported as number of genomes/100,000 WBC's. Infectious disease consultation will be obtained if EBV DNA levels are > 1000 genomes/100,000 WBC's.
5) Infectious disease consultation should be obtained for patients who develop a positive Galactomannan EIA test only after D+28.
6) Patients should receive acyclovir or valacyclovir through day +365 according to institutional standard practice guidelines as prophylaxis for HSV and VZV. Acyclovir or valacyclovir can be withheld during periods that the patient is receiving ganciclovir, foscarnet or other effective antiviral drug for management of CMV.
7) After engraftment, patients should receive prophylaxis for pneumocystis carinii according to institutional standard practice guidelines.

12C. Ursodeoxycholic acid (UDCA) prophylaxis of hepatic complications of transplant

1. UDCA should be given to prevent hepatic complications according to standard practice guidelines. Specifically UDCA should be given from approximately 2 weeks prior to the start of conditioning until day +90 post transplant. If a patient develops GVHD continuation of UDCA beyond day +90 may be warranted at the discretion of the attending physician. The recommended dose of urosodeoxycholic acid (UDCA) is 12 mg/kg/day po divided in 2-3 daily doses with food.

12D. Management of acute and chronic GVHD

1. Patients who develop ≥ grade II acute GVHD should be treated with systemic corticosteroids and with beclomethasone or budesonide if clinically indicated. Patients failing initial therapy will be eligible for second line therapy. Second line immunosuppressant therapy includes but is not limited to sirolimus, mycophenolate mofetil, monoclonal antibodies, pentostatin, denileukin difitox, thalidomide and extracorporeal photopheresis, given on or off study protocols.

2. Patients who develop chronic GVHD should be treated with immunosuppressive therapy according to institutional guidelines or may be treated on research protocols.

12E. Management of Delayed Engraftment

1. If the ANC has not reached 100 by Day 21, a bone marrow examination may be performed to ascertain cellularity. If the ANC is <100 on Day 21, G-CSF, GM-CSF or other appropriate cytokines may be utilized and chimerism studies should be performed. Care of patients that have continued poor graft function after cytokine administration or who reject their grafts after initial engraftment is at the discretion of the attending physician.

12F. Management of Relapse

1. Patients who have persistent leukemia or who relapse after transplant may be treated according to institutional practice which can include but are not limited to chemotherapy and/or donor lymphocyte infusions (DLI), or may be eligible for research protocols evaluating immunotherapy with antigen-specific T cells.

13. Records

The medical record containing information regarding treatment of the patient will be maintained as a confidential document, within the guidelines of the Fred Hutchinson Cancer Research Center, Seattle Children's, the University of Washington Medical Center, the Seattle Cancer Care Alliance, Yale University School of Medicine and the Yale-New Haven Hospital.

Each patient is assigned a unique patient number to assure patient confidentiality. Patients should not be referred to by this number, by name, or by any other individual identifier in any publication or external presentation. The Clinical Statistics Department maintains a patient database at FHCRC to allow storage and retrieval of patient data collected from a wide variety of sources. The licensed medical records departments, affiliated with the institution where the patient receives medical care, maintains all original inpatient and outpatient chart documents.

The primary research records will be contained and accessed through CORE, an encrypted, password-protected web site maintained by the FHCRC Clinical Research Data Systems division. Access is restricted to personnel authorized in writing by the FHCRC principal investigator.

For Yale patients, primary research data may also be collected and maintained by the data coordinator and stored at the Yale Cancer Center Clinical Trials Office (CTO) in a locked room. Research records will only be accessible to research personnel involved in the study or those performing auditing functions.

Information gathered from this study regarding patient outcomes and adverse events will be made available to the sponsor and the Federal Drug Administration. All precautions to maintain confidentiality of medical records will be taken.

14. Evaluation and statistical considerations

14A. Type of study

This is a prospective phase II study of allogeneic stem cell transplantation using PBSC that are selectively depleted of $CD45RA^+$ T cells.

14B. Definition of endpoints

1. Acute GVHD
   a. Definition: Acute GVHD will be diagnosed and graded using the clinical and laboratory criteria in Appendix E. Acute GVHD is defined operationally as the occurrence of compatible symptoms or signs in the skin, gastrointestinal tract, or liver prior to Day +100. In most cases histology of biopsy material of at least one involved organ will be used to confirm acute GVHD, but biopsy is not absolutely required for the diagnosis.
   b. Evaluation: During the inpatient stay, clinical evaluations will occur daily.

During the outpatient stay, each patient should be evaluated at least weekly until day +30 and then at least every other week until departure. Clinical evaluations will be performed by an attending physician. Cutaneous, hepatic and gastrointestinal GVHD should be confirmed by biopsy except where medically contraindicated. An attending pathologist will interpret the biopsy material. The decision to initiate GVHD therapy will be made by the attending physician.
At the conclusion of the study, two pathologists with experience in GVHD will independently review the histology of biopsy specimens. An acute GVHD grade will be assigned for each patient by three experienced transplant physicians, one from each study site and one from a transplant center not involved in the study.

c. Endpoints for acute GVHD that will be collected include:
   i. Presence of acute GVHD grades II-IV
   ii. Time to onset of GVHD
   iii. Requirement for methylprednisolone-equivalent dose of >=0.75mg/kg at day 28
   iv. Cumulative dose of methylprednisolone equivalent during the first eight weeks
   v. Requirement for secondary systemic therapy for acute GVHD

2. Graft failure
   a. Definition: Graft failure is defined operationally as:
      i. Failure to reach an ANC of >500/μl for 3 consecutive days by day 28
      ii. Irreversible decrease in ANC to <100 after an established donor graft: If the reduction in ANC is the result of relapse, as determined by histopathology, flow cytometry or molecular studies, this will not be considered graft failure. If there is a reasonable explanation, such as viral infection or drug effect that may be responsible for a reversible decrease in ANC, this will be not be considered graft failure.
   b. Evaluation: Engraftment endpoints will include:
      i. Time to ANC of >500/uL on the first of three consecutive days.
      ii. Time to ANC of >1,000/uL on the first of three consecutive test results
      iii. Time to RBC transfusion independence.
      iv. Time to platelet count >20,000/μL for 3 days without transfusion.
      v. Time to platelet count >50,000/μL for 3 days without transfusion.
      vi. Chimerism analysis of CD3 and CD33 cells in peripheral blood performed on or around day 28, 56, 80-100, 180 and 360. Additional peripheral blood or marrow chimerism studies may be performed as part of the evaluation of cases of graft failure.
3. Relapse
   a. Definition: Relapse is defined by the presence of malignant cells in marrow, peripheral blood, or extramedullary sites by histopathology. Minimal residual disease is defined as the presence of malignant cells in the marrow, peripheral blood, or extramedullary sites detectable only by molecular methods, cytogenetics, or flow cytometry, but not observed by histopathology.
   b. Evaluation: Testing for recurrent malignancy in the blood and bone marrow will be performed by monitoring the CBC and bone marrow as outlined in Table 5. Suspected extramedullary sites of recurrent disease should be evaluated by biopsy and/or lumbar puncture if clinically indicated.
4. Chronic GVHD
   a. Definition: Chronic GVHD will be diagnosed using NIH criteria outlined in

Appendix F.

b. Chronic GVHD will be defined operationally as the occurrence of compatible symptoms. Evaluation: The primary chronic GVHD endpoint will be the occurrence of chronic GHVD requiring systemic immunosuppression. Additional chronic GVHD endpoints may include:
   i. Severity of GVHD – Mild versus Moderate versus Severe.
   ii. Duration of immune suppression (other than the standard tacrolimus taper) in months after diagnosis.
   iii. Number of cycles of immune suppressant therapy. A cycle is defined as either a nine-month course of a calcineurin inhibitor and prednisone given according the Long Term Follow-Up standard policy at the FHCRC or a course of any second-line immunosuppressant therapy given on or off protocol.
   iv. Use of additional immune suppressive agents other than first line therapy (prednisone and tacrolimus/CSP).

5. Transplant related mortality (TRM). TRM is defined as mortality in any patient for whom there has not been a diagnosis of relapse.

14C. Statistical Analysis and Stopping Rules

1. Acute GVHD

The primary objective of this single-arm Phase II study is to estimate the probability of grades II-IV acute GVHD and compare this to relevant historical experience. The goal is to observe a statistically significant reduction in the probability of GVHD compared to this historical experience without an accompanying increase in the probability of graft failure. Primary endpoints are grades II-IV acute GVHD and graft failure. Secondary endpoints include grades III-IV acute GVHD, relapse, transplant-related mortality, rate of engraftment, and chronic GVHD. Based on the historical experience at the FHCRC with unmanipulated PBSC grafts for 74 patients who received a first transplant from a matched related donor on protocols 179, 739, and 1106 since 2001, the probability of grade II-IV acute GVHD is 61%, and the probability of grade III-IV acute GVHD is 19%. The rates of grade II-IV and grade III-IV acute GVHD were 62% and 17% respectively, for 29 AML and ALL patients who received unmanipulated PBSC grafts from HLA-matched related donors and received post transplant immunosuppression with tacrolimus and methotrexate on Protocol 1932. Based on these data, we shall use a rate of grade II-IV acute GVHD of 60% as our benchmark for comparison in the current trial. Table 6 summarizes the number of evaluable patients needed in the current trial to achieve 90% power to observe a statistically significantly (at the one-sided level of .05; power estimated from chi-square test) reduced probability of grades II-IV acute GVHD compared to this fixed value for various assumed-true probabilities.

Table 6

Fixed Historical Probability	Assumed-true Probability, CD45RA depleted	Number Patients
.60	.40	52
.60	.35	33
.55	.30	22

Based on these numbers, we propose to enroll 35 evaluable patients in the first cohort of patients on the current trial (which will provide 92% power if the assumed-true

GVHD rate CD45RA-depleted patients is 35%). If 15 or fewer of the 35 patients (43% or less) develop grades II-IV acute GVHD, then the probability of GVHD will be considered to be sufficiently low to proceed with the second cohort of patients. The probability of this occurring if the true probability of grades II-IV GVHD is .60 is .03. Such an occurrence would therefore give us high confidence that the true GVHD rate is less than the benchmark of 60%. GVHD will be treated as a binary endpoint, and as such, patients who do not develop GVHD will be regarded as non-failures for purposes of evaluating GVHD, regardless of whether they died or survived to day 100 without GVHD. If 15 or fewer patients develop grades II-IV acute GVHD and the limits for graft failure (14.C.2) are not met, a second cohort of 25 patients will be treated with a shorter course of tacrolimus in an effort to see if the reduced GVHD rate can be maintained with a further reduction in immunosuppression. This study will not be capable of proving statistically that the GVHD rate with reduced immunosuppression is no worse than it is with the immunosuppression regimen utilized in the initial cohort of 35 patients. We propose to enroll 25 patients with short course tacrolimus, and if 10 or fewer (40% or less) experience grades II-IV acute GVHD the reduced immunosuppression will be considered promising and worthy of further study. If the true probability of grades II-IV acute GVHD is 0.50, then the probability of observing 10 or fewer cases of grades II-IV GVHD among 25 patients is 0.21.

Modification April 2014

An interim analysis of protocol 2222 was performed after the first 30 patients were treated with a median of 592 days follow-up for surviving patients. In summary, an excellent rate of engraftment (a primary endpoint), a very low (12%) rate of chronic GVHD (a secondary endpoint), low rates of relapse and non-relapse mortality (secondary endpoints), very good immune reconstitution and survival were observed. In the first 30 patients acute GVHD is not lower than the historical benchmark (primary endpoint). However, compared to the first 13 naïve T cell-depleted HCT recipients (85%), acute GVHD appears to be lower among 17 subsequent patients (14-30) (53%) who received antibiotic prophylaxis directed at gram positive organisms (due to a high rate of streptococcus viridans bacteremia amongst the first 13 patients). The original plan was to enroll 35 patients with naïve T cell depletion and tacrolimus monotherapy and if acute GVHD was reduced in this cohort then to enroll a second cohort of patients without tacrolimus. Although the desired reduction in acute GVHD will not be achieved in the first 35 patients, the chronic GVHD rates are very low, the general outcomes are favorable and it is still possible that acute GVHD will in fact be reduced among patients who receive naïve T cell depletion and penicillin.

We acknowledge that through the first 30 patients enrolled to this trial attainment of our primary endpoint is not possible (15 or fewer cases of grades 2-4 acute GVHD among 35 patients). We further acknowledge that the original version of the protocol did not have stated as a primary or secondary endpoint the probability of grades 2-4 acute GVHD among patients who received penicillin for infection prophylaxis. Given the observation that patients who received penicillin might have a lower rate of grades 2-4 acute GVHD (the primary endpoint of this Phase II trial), we are extending accrual to 2222 to an intended sample size of n=35 patients who will receive penicillin. The same design considerations will hold for the "penicillin patients" as were in place in the original protocol. We acknowledge that this will lead to a bias in

the estimate of our primary endpoint, as we plan to include the 17 patients already enrolled and treated with penicillin in our analysis since their outcomes have already been observed (9/17 (53%) patients with grades 2-4 acute GVHD). However, this observed rate is closer to the null hypothesis (GVHD probability of 60%) than the alternative, so under the alternative hypothesis that the probability of GVHD is 35%, the bias will be positive relative to 35% (in fact, based on a simulation study, the bias under alternative hypothesis is roughly 8%; under the null hypothesis, the bias is approximately 2%). And while it was not intended as such, and we fully acknowledge this, one might loosely think of the first 17 patients who received penicillin as the first stage in at two-stage design, after which the trial could be terminated if "too many" cases of GVHD are observed. Towards that end, the probability of 9 or more cases of GVHD among 17 patients under the null hypothesis of 35% is .10; under the assumption that the true probability of GVHD is 40%, the probability of 9 or more cases of GVHD among 17 patients is .20. Therefore, if this truly were the first stage of a two-stage trial, we would not have enough evidence to reasonably terminate the trial. While the power to detect a statistically significant difference relative to the fixed benchmark of 60% is certainly lower with outcomes already observed for roughly half the patients, the conditional power (given 9/17 already observed) under the alternative hypothesis of 35% GVHD is 55% (at the one-sided significance level of .05; at the one-sided significance level of .10, the conditional power is 73%. Given the observed outcome of 9/17, the probability of "success" after 35 patients (15 or fewer cases of GVHD, or 6 or fewer among the next 18) under then null hypothesis is p=.02 (i.e., the type I error rate). The protocol will be terminated if it becomes apparent that the primary endpoint will not be met among the penicillin patients. In other words, if a 16^{th} case of grades 2-4 acute GVHD occurs among the 35 patients treated with penicillin (or a 7^{th} case among the additional 18 patients post-modification), then success for our primary endpoint will not be attainable and the study will be terminated.

It is theoretically plausible, although unlikely, that severe, steroid refractory GVHD could be increased even though T-cells are being removed. Because of this, we will have in place stopping rules for this outcome. These rules will be separately applied for each cohort. If there is sufficient evidence to suggest that the true probability of steroid refractory GVHD exceeds 5%, the study will be stopped. Sufficient evidence will be taken to be an observed rate whose lower one-sided 90% confidence limit exceeds 5%. Operationally this will occur if any of the following observed ratios occur: 2 of the first 10 or fewer, 3 of the first 22 or fewer, or 4 of the first 35 or fewer patients develop steroid refractory GVHD. If the true probability of GVHD is 0.03, the probability of stopping after 20 or 35 patients is approximately 0.05 and 0.06, respectively. If the true probability is 0.23, then the probability of stopping is approximately 0.90 and 0.98, respectively. The presence of steroid refractory GVHD will be defined by the decision of the attending physician to initiate secondary systemic therapy for acute GVHD due to concerns that corticosteroids are providing inadequate control of acute GVHD manifestations.

2. Graft Failure

Graft failure will be closely monitored throughout this study. A true probability of graft failure over 5% will be considered excessive. As with GVHD, graft failure will be treated as a binary outcome. If two graft failures are seen among the first 16

patients, 3 among the first 30 patients, or 4 among the first 35 patients enrolled the trial will be stopped due to excessive graft failure. These limits correspond to observed rates of graft failure whose lower one-sided 80% confidence limits exceed 5%. Demanding higher confidence that the estimated graft failure rate exceeds 5% before stopping means that the chances the trial would stop when the true graft failure rate is over 5% are unacceptably low. If the true failure rate is as low as 1%, then the probability that the trial stops after 20 or 35 patients is approximately .01, and .01, respectively. If the true failure rate is 16%, these probabilities are .78 and .91, respectively.

These stopping rules for the modification will be applied to the group of patients receiving penicillin.

Graft failure will continue to be closely monitored in the second cohort of 25 patients. Since shortening the tacrolimus post transplant should not increase the probability of graft failure, the experience of the initial group of 35 patients will be considered when assessing graft failure. Extending the stopping rules for graft failure listed above, 4 failures among the first 46 patients or 5 among the first 60 patients will be considered excessive. If the true probability of graft failure is .01, the probability of stopping after 45 or 60 patients is approximately .02 and .02, and if the true probability of failure is .16 the probability of stopping after 45 and 60 patients is approximately .96 and .99, respectively.

3. Relapse
   Relapse will also be carefully monitored. While formal stopping rules will not be put in place for relapse due to the relatively long period of time post-transplant that this complication is of concern, a Data Safety and Monitoring Board (DSMB) will be in place to meet roughly every 6 months (or whenever the last patient in each group of five patients reaches day 100, whichever is sooner) to review the data. This DSMB will be composed of Drs' Eneida Nemecek, Elihu Estey, Soheil Meshinchi, Blythe Thomson and Barry Storer. Rough guidelines for acceptable rates of relapse will be 50% for patients transplanted in relapse and 25% for patients transplanted in remission.

4. Failure to deliver therapy
   If CD45RA-depleted PBSC cannot be delivered as planned for the first 2 patients enrolled, the study will be suspended for a period of time in order to optimize the procedure. This is defined as failure to deliver a minimum of 2.0×10^6 $CD34^+$ cells/kg recipient body weight. If an improved procedure is achieved, the study may be resumed. This rule will be reinstated for the subsequent enrollment.

5. Patient withdrawal from treatment or study
   All patients who receive infusion of PBSC with or without cell selection will be considered evaluable for study endpoints. Patients in whom tacrolimus is discontinued, or switched to cyclosporin, due to concerns for toxicity will still be considered on study.

   When a patient withdraws consent from P2222 they will no longer have research samples taken and the research team will no longer have access to clinical records to

follow them for study endpoints.

The only exception is for FHCRC patients who have signed the general consent and authorization form allowing their leftover specimens and medical records to be used for research. If the FHCRC patient has signed that consent then they will still be evaluable for clinical endpoints although research blood draws would be discontinued. If the patient refused to sign this general consent or withdrew general consent to the use of clinical data for research purposes then no further follow-up would be done.

15. Guidelines for Reporting and Tracking Events

15A. Toxicity Grading

Toxicities will be graded according to the current version of the NCI Common Terminology Criteria for Adverse Events (CTCAE) Version 4. The full text of the NCI CTCAE is available online at:

http://evs.nci.nih.gov/ftp1/CTCAE/About.html

15B. Definitions

Definitions associated with reportable events can be found on the FHCRC's Institutional Review Office (IRO) extranet website (Table 7 FHCRC IRB Forms for Reporting).

Unanticipated adverse device effect means any serious adverse effect on health or safety or any life-threatening problem or death caused by, or associated with, a device, if that effect, problem, or death was not previously identified in nature, severity, or degree of incidence in the investigational plan or application (including a supplementary plan or application), or any other unanticipated serious problem associated with a device that relates to the rights, safety, or welfare of subjects. (21 CFR 812.3)

15C. Tracking and Reporting of Events

Patients enrolled in this study are receiving treatments that are generally associated with high rates of "expected" adverse events (outlined in Appendix G as well as Section 11 of the protocol). The following events will be tracked and reported:

1. Non-hematologic adverse events assessed as Grade 3-5 per NCI CTCAE, expected or unexpected, from the start of study treatment (pre-transplant conditioning) through day 100 will be collected.
2. Grade 3-5 Blood/Bone Marrow adverse events occurring between day 43 and day 100 will be collected.
3. Graft versus host disease assessment done as part of routine care will be reviewed approximately once weekly through day 100. Details about GVHD symptoms, diagnosis, treatment, and outcome will be collected. After day +100, GVHD data will be captured at day +180, 1 year, and then yearly through 5 years after day 0 whenever possible.
4. Relapse, graft failure, and death data will be captured as they occur in the first 200 days post transplant for all patients, and from 200 days up to 5 years whenever possible. All treatment related mortality occurring in the first 100 days will be reported to all parties in an expedited fashion.
5. Grade 3-5 infusion reactions and grade 4-5 thrombotic microangiopathy (TMA) occurring in the first 100 days will be reported to all parties in an expedited fashion.

15D. Reporting to Coordinating Center

As FHCRC is the coordinating center reportable events occurring at the FHCRC and participating sites will be collected by the FHCRC PI and/or study nurse and reported to FHCRC IRB. Reportable events occurring in YUSM patients will be reported by the YUSM investigator or representative to the FHCRC PI and/or study nurse. The trial coordinators at collaborating centers or the local PIs will verbally report all events requiring expedited reporting (including, but not limited to, all patient deaths regardless of cause, occurring days 0-200 post-transplant procedure) within 72 hours of learning of the event and an official report of the event is faxed to the coordinating center (FHCRC) at 206-667-6056 within seven days utilizing the FHCRC Adverse Event Reporting Form and the FHCRC Expedited Reporting Form for Unanticipated Problems or Noncompliance. Follow-up information to a reportable event report must be submitted as soon as the relevant information is available.

The FHCRC PI and research nurse will meet regularly to review all reported events. If the event meets FHCRC IRB current reporting obligations it will be sent to them.

After the FHCRC IRB has reviewed the event report it will be disseminated to all participating sites/investigators, the DSMB, and the study sponsor.

15E. Reporting Requirement to FHCRC IRB

The Principal Investigator, study nurse, or coordinator shall submit to the FHCRC IRB reportable events occurring in Cancer Consortium patients and multi-center sites according to current reporting policies as outlined in Table 7 FHCRC IRB Policies for Reportable Events.

As FHCRC is the coordinating center reportable events occurring at the FHCRC and participating sites will be collected by the FHCRC PI and/or study nurse and reported to FHCRC IRB.

All reportable events should be submitted on the relevant FHCRC Forms (URLs linking to the FHCRC IRO website are found in Table 8 FHCRC IRB Forms for Reporting).

Table 7 FHCRC IRB Policies for Reportable Events.

(Relevant FHCRC policies include, but are not limited to the following documents. Please also refer to the FHCRC IRO website.)

IRB Policy 2.6	Adverse Events and Other Unanticipated Problems Involving Risks to Subjects or Others	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.9	Noncompliance with the Office of the Director's Human Research Protection Program Policy	http://extranet.fhcrc.org/EN/sections/iro/irb/ae.html
IRB Policy 1.1	Reporting Obligations for Principal Investigators	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html
IRB Policy 2.2	Continuing Review	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html
IRB Policy 1.13	Investigational New Drugs (IND), Biologics and Investigational Device	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html

	Exemptions (IDE)	

Table 8 FHCRC IRB Forms for Reporting

Adverse Event Reporting Form	http://extranet.fhcrc.org/EN/sections/iro/irb/forms/index.html
Expedited Reporting Form for Unanticipated Problems or Noncompliance	http://extranet.fhcrc.org/EN/sections/iro/irb/forms/index.html

15F. Reporting to the Sponsor

Unanticipated adverse device events, grade 3-5 infusion reactions and grade 4-5 thrombotic microangiopathy (TMA) occurring in the first 100 days post-transplant will be reported and sent via email to the sponsor, Dr. Warren Shlomchik (warren.shlomchik@yale.edu) and the IDE coordinator, as soon as possible but no later than 10 working days of learning of the event. A confirmatory email communication must be received from at least one of these individuals. If a confirmation email is not received within 2 working days, Dr. Shlomchik and the IDE coordinator should be contacted again by email and additionally by telephone or FAX.

In addition, graft failure, death or grade 4 non-hematologic events not resolving to grade 2 or less within 96 hours occurring in the first 200 days post-transplant, will be reported to the sponsor of the IDE, Dr. Warren Shlomchik, as soon as possible but in no event later than 10 working days after the investigator first learns of the event.

15G. Reporting Requirement to Yale IRB

All reportable events whether originating at Yale or a collaborating center will be reported by Yale study staff to the Yale University Human Investigation Committee (HIC) using HIC Form 6A or the Protocol Deviation or Unanticipated Problem Involving Risks to Subjects or Others Report Form within 48 hours of Yale learning of the event. A copy of the HIC Adverse Event Policy is available at:
http://www.yale.edu/hrpp/policies/index.html#

A copy of the HIC Adverse Event and Deviation or Unanticipated Problem report form is available at:
http://www.yale.edu/hrpp/forms-templates/biomedical.html

15H. Reporting to the FDA

For studies conducted under an IDE (Investigation Device Exemption) the FDA regulations outline the following sponsor obligations regarding unanticipated adverse device effects:

A sponsor shall immediately evaluate any unanticipated adverse device effect. 21CFR812.46 (b) (1)

A sponsor who conducts an evaluation of a unanticipated adverse device effect under 21 CFR 812.46(b) shall report the results to FDA within 10 working days after the sponsor first receives notice of the effect. Thereafter the sponsor shall submit such additional reports concerning the effect as FDA requests. 21CFR812.150 (b) (1)

A **MedWatch 3500A** form will be completed and sent to the FDA

A sponsor who determines that an unanticipated adverse device effect presents an unreasonable risk to subjects shall terminate all investigations or parts of investigations presenting that risk as soon as possible. Termination shall occur not later than 5 working days after the sponsor makes this determination and not later than 15 working days after the sponsor first received notice of the effect. 21CFR812.46 (b) (2)

16. Data safety monitoring plan

16A. Monitoring the progress of trials and the safety of participants

The FHCRC PI and IDE sponsor are responsible for monitoring this multi-institutional clinical trial, with oversight by a Data and Safety and Monitoring Board (DSMB), the Protocol and Data Monitoring Committee (PDMC) at the FHCRC, the Yale Cancer Center Office of Protocol Review and Monitoring and the IRBs at the respective institutions. This is a Phase II study and the assessment of risk is considered above minimal. The PI reviews outcome data for each individual patient at approximately 3 months after HCT.

A DSMB will be in place to meet approximately every 6 months (or whenever the last patient in each group of five patients reaches day 100, whichever is sooner) to review the data particularly as it relates to engraftment, grades III-IV GVHD and relapse. The DSMB will also review all adverse events reported to the coordinating center (FHCRC). The DSMB confirms that the trial has not met any stopping rules and reviews any patient safety problems necessitating discontinuation of the trial. A report from the DSMB is submitted to the Coordinating Center IRB (FHCRC) and the PI. The FHCRC study staff will send a copy of the reports to the IDE sponsor, as well as to the trial coordinators/local Investigators' of this protocol. Each institution will then distribute this report per their internal policies. The DSMB will discontinue the review of outcomes when all subjects on this trial have completed all protocol-specified follow-up. The DSMB for protocol 2222 will be composed of Drs' Eneida Nemecek, Elihu Estey, Soheil Meshinchi, Rebecca Gardner, and Barry Storer.

P2222 DSMB Members

Name	Affiliation	Position
Dr. Eneida Nemecek	Oregon Health & Science University	DSMB Chair
Dr. Barry Storer	FHCRC	DSMB Biostatistician
Dr Soheil Meshinchi	FHCRC	DSMB Clinical Investigator
Dr. Elihu Estey	SCCA	DSMB Clinical Investigator
Dr. Rebecca Gardner	Seattle Children's Research Institute	DSMB Clinical Investigator

All members have experience in the management of patients with leukemia and myelodysplasia and in the conduct and monitoring of clinical trials.

The PDMC at FHCRC and the Quality Assurance Compliance and Safety Committee (QUACS) at Yale will review the progress of the protocol with respect to the monitoring plan at the time of each annual renewal. As with initial review, annual FHCRC IRB review and approval is also required. YUSM also has an IRB that will review and monitor the protocol. Each IRB will be contacted by the local Investigator in accordance with their IRB reporting guidelines.

An external monitor will be retained to monitor study progress at both the FHCRC and at Yale. The scope of monitoring will be based on the FHCRC/UW Data and Safety Monitoring Plan: http://www.cancerconsortium.org/rto/prr/DSMPlan.pdf . Per the DSMP subjects will be randomly selected for verification. An initial monitoring visit is expected within six months of enrollment of the first subject and preferred prior to enrollment exceeding 4 subjects as 100% verification is expected during an initial visit. Monitoring reports will be forwarded to the DSMB, the IDE sponsor and the PI at FHCRC. Each institution will then distribute this report per their internal policies.

Flow of information concerning clinical trial participants originates with the clinicians and nurses in the clinic or referring clinicians at other institutions and is transmitted to the FHCRC Research Nurse. At the FHCRC and YUSM, health care providers and rotating attending physicians assess patients and record their observations regarding toxicity and response outcomes in the medical record. Thus, multiple health care providers provide independent observations and participate in monitoring this trial. The PI may be a clinician for some patients entered on this trial. However, assessments are the sum total of the multiple clinicians involved with the patient averting possible conflict of interest having the PI as the attending clinician for protocol patients. If determination of adverse events is controversial, co-investigators will convene on an ad hoc basis as necessary to review the primary data and render a decision.

16B. Plans for assuring data accuracy and protocol compliance

The study has a research nurse at each site that follows patients to confirm eligibility, reporting of adverse events, reporting of events which are part of the safety-monitoring plan, and protocol adherence. Each site's investigator and research nurse are responsible for review and maintenance of all patient research records to ensure data integrity and protocol adherence.

Health care providers and rotating attending physicians assess patients and record their observations in the medical record. This documentation is extracted by the site's research staff by approximately day 100 (and no later than day +180) and again approximately yearly for 5 years after HCT via chart review and entered into electronic/paper based protocol specific Case Report Forms (CRFs). The principal investigator at each site will review the CRFs and the primary source documents verifying by signature (paper/electronic) their data accuracy.

The Coordinating Center PI will review and send queries to the collaborating investigator if CRFs are incomplete or inaccurate.

The study is monitored under the FHCRC Monitoring Plan. The FHCRC Data and Safety Monitoring Plan details the full scope and extent of monitoring and provides for immediate action in the event of the discovery of major deviations.

17. References

1. Ferrara JL, Reddy P. Pathophysiology of graft-versus-host disease. Semin Hematol 43:3-10, 2006.
2. Bensinger WI, Martin PJ, Storer B, et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients with hematologic cancers. N Engl J Med 344:175-81, 2001.
3. Oehler VG, Radich JP, Storer B, et al. Randomized trial of allogeneic related bone marrow transplantation versus peripheral blood stem cell transplantation for chronic myeloid leukemia. Biol Blood Marrow

Transplant 11:85-92, 2005.

4. 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 92:2303-14, 1998.
5. Chao NJ. Minors come of age: Minor histocompatibility antigens and graft-versus-host disease. Biol Blood Marrow Transplant 10:215-23, 2004.
6. Champlin RE, Passweg JR, Zhang MJ, et al. T-cell depletion of bone marrow transplants for leukemia from donors other than HLA-identical siblings: advantage of T-cell antibodies with narrow specificities. Blood 95:3996-4003, 2000.
7. Ho VT, Soiffer RJ. The history and future of T-cell depletion as graft-versus-host disease prophylaxis for allogeneic hematopoietic stem cell transplantation. Blood 98:3192-204, 2001.
8. Goodnow CC, Sprent J, Fazekas de St Groth B, Vinuesa CG. Cellular and genetic mechanisms of self tolerance and autoimmunity. Nature 435:590-7, 2005.
9. Chao NJ, Chen BJ. Prophylaxis and treatment of acute graft-versus-host disease. Semin Hematol 43:32-41, 2006.
10. Storb R, Deeg HJ, Whitehead J, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. N Engl J Med 314:729-35, 1986.
11. Brown JM. Exogenous administration of immunomodulatory therapies in hematopoietic cell transplantation: an infectious diseases perspective. Curr Opin Infect Dis 18:352-8, 2005.
12. Papadopoulos EB, Carabasi MH, Castro-Malaspina H, et al. T-cell-depleted allogeneic bone marrow transplantation as postremission therapy for acute myelogenous leukemia: freedom from relapse in the absence of graft-versus-host disease. Blood 91:1083-90, 1998.
13. Jakubowski AA, Small TN, Young JW, et al. T-cell depleted transplantation for adults with hematologic malignancies: sustained engraftment of HLA-matched related donor grafts without the use of anti-thymocyte globulin. Blood 110:4552-9, 2007.
14. Keever CA, Small TN, Flomenberg N, et al. Immune reconstitution following bone marrow transplantation: comparison of recipients of T-cell depleted marrow with recipients of conventional marrow grafts. Blood 73:1340-50, 1989.
15. Lanzavecchia A, Sallusto F. Dynamics of T lymphocyte responses: intermediates, effectors, and memory cells. Science 290:92-7, 2000.
16. Kaech SM, Wherry EJ, Ahmed R. Effector and memory T-cell differentiation: implications for vaccine development. Nat Rev Immunol 2:251-62, 2002.
17. Anderson BE, McNiff J, Yan J, et al. Memory CD4+ T cells do not induce graft-versus-host disease. J Clin Invest 112:101-8, 2003.
18. Zhang Y, Joe G, Zhu J, et al. Dendritic cell-activated CD44hiCD8+ T cells are defective in mediating acute graft-versus-host disease but retain graft-versus-leukemia activity. Blood 103:3970-8, 2004.
19. Zheng H, Matte C, Anderson BE, et al. CD8+ central memory T cells mediate graft-versus-host disease although retain graft-versus-leukemia effect. Blood 106:2005.
20. Chen BJ, Cui X, Sempowski GD, Liu C and Chao NJ. Transfer of allogeneic CD62L- memory T cells without graft-versus-host disease. Blood 103:1534-41, 2004.
21. Zheng H, Matte-Martone, Hongmei L, et al. Effector memory CD4+ T cells mediate graft-versus-leukemia without inducing graft versus host disease. Blood 111: 2476-84, 2008.
22. Xystrakis E, Bernard I, Dejean AS, Alsaati T, Druet P, Saoudi A. Alloreactive CD4 T lymphocytes responsible for acute and chronic graft-versus-host disease are contained within the CD45RChigh but not the CD45RClow subset. Eur J Immunol 34:408-17, 2004.
23. Arstila TP, Casrouge A, Baron V, Even J, Kanellopoulos J, Kourilsky P. A direct estimate of the human alphabeta T cell receptor diversity. Science 286:958-61, 1999.
24. Burrows SR, Khanna R, Silins SL, Moss DJ. The influence of antiviral T-cell responses on the alloreactive repertoire. Immunol Today 20:203-7, 1999.
25. Bleakley M, Mollerup A, Chaney C, Brown M, Riddell S. Human minor histocompatibility antigen-specific CD8+ T cells are found predominantly in the CD45RA+ CD62L+ Naive T cell subset. Blood 106:2005.
26. Smalley DM, Ley K. L-selectin: mechanisms and physiological significance of ectodomain cleavage. J Cell Mol Med 9:255-66, 2005.
27. 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 96:2062-8, 2000.
28. Uberti JP, Ayash L, Braun T, Reynolds C, Silver S, Ratanatharathorn V. Tacrolimus as monotherapy or

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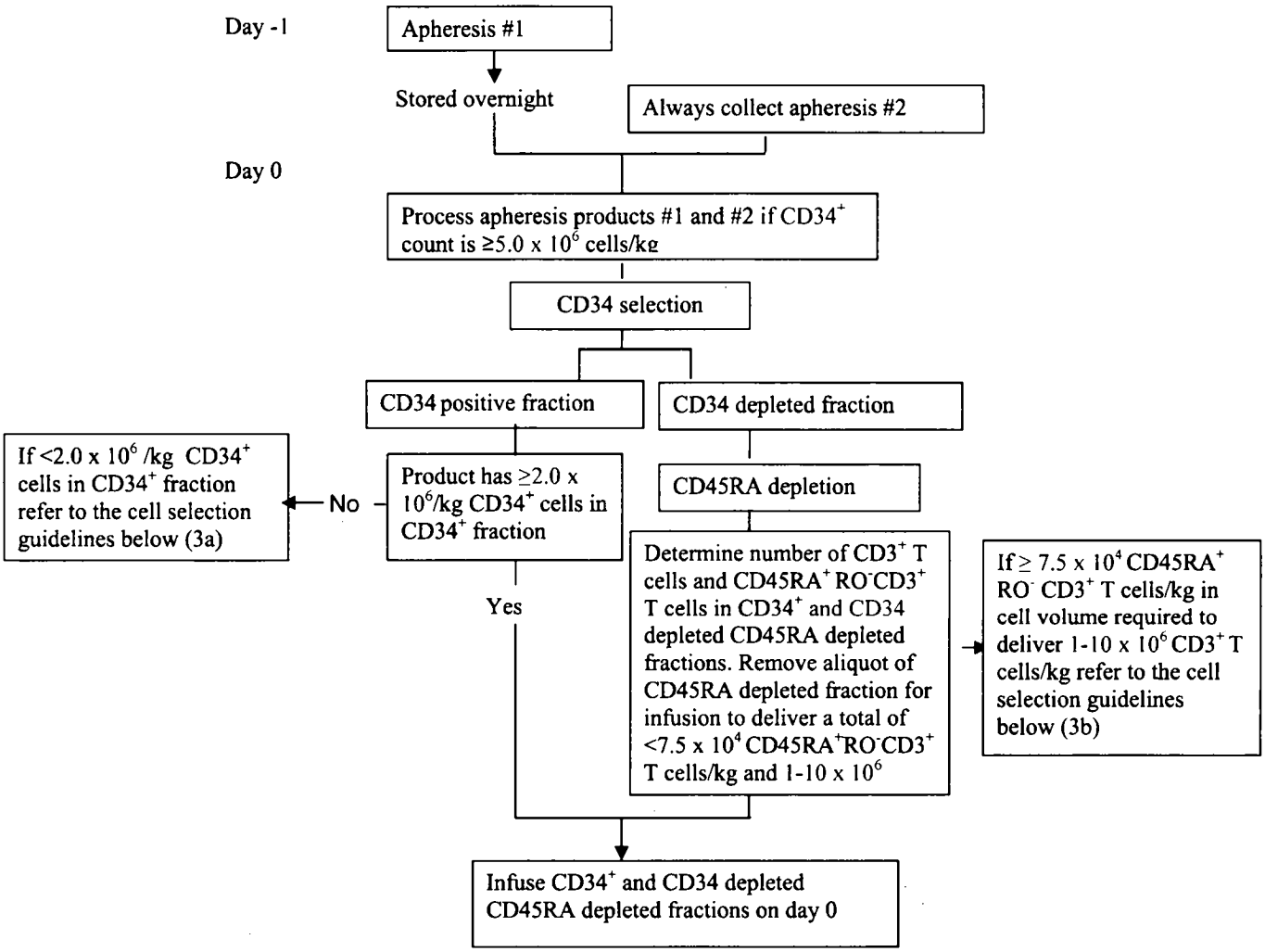
combined with minidose methotrexate for graft-versus-host disease prophylaxis after allogeneic peripheral blood stem cell transplantation: long-term outcomes. Bone Marrow Transplant 34:425-31, 2004.

APPENDIX A

Cell Selection Flow Sheet



Based on prior experience with cell selection of PBSC, we expect the following:

- A total CD34 cell dose of $\geq 5x10^6$ cells/kg of recipient body weight will be available from two donor 12-16L leukapheresis collections for 85% of donors. A total $CD34^+$ cell dose of $>2.0x10^6$ cells/kg of recipient body weight will be obtained from CD34 positive selection from an apheresis product containing $5x10^6$ $CD34^+$cells/kg of recipient body weight.
- The number of residual donor $CD45RA^+RO^-CD3^+$ T cells in the CD34 selected product that will contribute to the overall dose of $CD45RA^+RO^-CD3^+$ T cells will be $<4.2.x10^4$/kg of recipient body weight.
- Depletion of $CD45RA^+$ cells from the CD34 negative fraction will yield sufficient T cells to administer 1-10 $x10^6$ $CD3^+$ cells/kg and $<3x10^4$ $CD45RA^+$ RO^-CD3^+ cells/kg of recipient body weight for >90% of patients.
- The maximum number of $CD45RA^+$ RO^-CD3^+ cells anticipated in the sum of the CD34 selected product and the CD45RA depleted product will be 7.5 x 10^4 $CD45RA^+RO^-CD3^+$ T cells/kg.

Cell Selection Guidelines

1. We will perform cell selections only when the PBSC product from days -1 and 0 combined contains $\geq 5x10^6$ $CD34^+$ cells/kg of recipient body weight. If the PBSC product from days -1 and

0 combined contains <5x10^6 $CD34^+$cells/kg of recipient body weight the PBSCs will be infused without any cell selection and the patients will not be included in the analysis of study efficacy end points. The patients that receive G-PBSC that does not undergo cell selection will receive GVHD prophylaxis with tacrolimus and methotrexate (see section 9F).

2. Priority will be given to administering adequate numbers of $CD34^+$ cells to achieve reliable engraftment. In most cases we expect to infuse 5-10 million $CD34^+$ cells/kg. The infused $CD34^+$ cell number may be limited to 10 million $CD34^+$ cells/kg at the PI's discretion or less (within the range of 2-10 million $CD34^+$ cells/kg) if necessary to achieve the limit of $\leq 7.5 \times 10^4$ $CD45RA^+CD45RO^-CD3^+$ cells/kg.
3. Situations may arise where the goals for cell selection are not achieved with processing of the two leukapheresis collections. These unusual situations will be discussed with the protocol PI and a recommendation made. General guidelines are as follows:
    a) *If* a total CD34 cell dose of $\geq 2.0 \times 10^6$ cells/kg is *not* achieved after processing of two leukapheresis collections all available $CD34^+$ cells will be administered to the patient on day 0 and a third apheresis collection will be performed on day 1 and the un-manipulated G-PBSC product will be administered to the patient without any cell selection procedure. These patients will not be included in the analysis of study efficacy end points and will receive GVHD prophylaxis with tacrolimus and methotrexate (see section 9F).
    b) If $< 7.5 \times 10^4 CD3^+$ $CD45RA^+$ RO^- cells/kg in the sum of the $CD34^+$ fraction and CD45RA depleted fraction cannot be achieved by simply reducing the number of $CD3^+$ and/or $CD34^+$ cells within the target range (minimum of 2.0×10^6/kg $CD34^+$ cells and a range of $1\text{-}10 \times 10^6$/kg total $CD3^+$ T cells) as described above, the following steps may be required:
        i. Repeat the CD45RA depletion of the CD34 depleted fraction using a second magnetic column.
        ii. If there is $>5 \times 10^4$ $CD3^+$ $CD45RA^+$ RO^- cells in the $CD34^+$ fraction and $>7.5 \times 10^6$ $CD34^+$ cells/kg in the $CD34^+$ fraction, a second column pass of an aliquot of the $CD34^+$ cells may be performed but a minimum of 2.0×10^6 $CD34^+$ cells/kg MUST be maintained.
        iii. If 1×10^6 $CD3^+$ cells/kg and $<7.5 \times 10^4 CD3^+CD45RA^+$ RO^- cell/kg cannot be achieved by i. or ii. then the patients should receive the CD34 selected product (minimum of 2.0×10^6 $CD34^+$ cells/kg) and may receive an aliquot of the $CD3^+$ cells (i.e. less than 1×10^6 $CD3^+$ cells/kg) containing $<7.5 \times 10^4 CD3^+CD45RA^+$ RO^- cell/kg. These patients who do not meet the goals for cell selection will not be included in the analysis of study end points. Patients will receive immunosuppression with tacrolimus as described in the protocol unless they receive only the $CD34^+$ selected product and $< 1 \times 10^5$ $CD3^+$ cells/kg in which case they will receive no immunosuppression.

APPENDIX B
Product Testing

The CD34-enriched and CD45RA-depleted cells, because they are derived solely as the result of a cell selection process with no culturing required, will be considered as having undergone "minimal manipulation". Each of the CD34-enriched and CD45RA-depleted cell batches will be tested for safety, purity and potency, identity and stability as indicated below. In general, once myeloablative therapy has been initiated, the patient must receive the CD34-selected PBSC product, to reconstitute his/her hematopoietic system.

a) Safety: Samples from the final products will be sent for sterility testing for bacterial and/or fungal contamination of products according to methods specified in 21 CFR 610.12. The tests will be performed in clinical laboratories affiliated with each cell processing center, according to validated procedures. Samples will be inoculated into thioglycollate broth and/or agar media. Media will be incubated at appropriate temperatures, and inspected for bacterial and/or fungal growth over a period of 14 days. Subculture onto agar plates will be performed to investigate any turbidity noted in broth cultures, and if positive cultures are found organism species identification will take place. Each institution has defined procedures and action plans in place to notify appropriate personnel and take appropriate measures if positive cultures are detected after infusion has taken place. After final product processing a sample will be pulled and a rapid gram stain will be performed with an acceptance criterion of "No Organisms Detected". If there is a positive gram stain, the attending physician will be notified. The attending physician will decide whether to administer the product, and if infused whether to begin prophylactic antibiotics. If the product is administered, we would await product culture results and if positive, modify antibiotic therapy accordingly and proceed as per Appendix C.

b) Purity and potency:
$CD34^+$ product: Viability will be determined by flow cytometry using exclusion of propidium iodide or 7-AAD with a notification specification set of >70% viable cells (i.e. If there are <70% viable cells in the $CD34^+$ product the PI will be notified). Quantitation of $CD34^+$ cells will be performed by flow cytometry, with a notification specification of >70% final purity and $CD34^+$ cell dose of >= 2.0×10^6 $CD34^+$ cells/kg. Quantitation of residual naïve T-cells ($CD3^+CD45RA^+CD45RO^-$) and total T cells ($CD3^+$) will be performed by multi-color flow cytometry, however no specification will be set for these cell subsets other than the total dose indicated below.

$CD45RA^-$ product: Viability will be determined by flow cytometry using exclusion of propidium iodide or 7-AAD, with a notification specification set of >70% viable cells. Quantitation of residual naïve T-cells ($CD3^+CD45RA^+CD45RO^-$), and total T cells ($CD3^+$) will also be performed by multi-color flow cytometry, The aim is to infuse a maximum of 7.5×10^4 $CD3^+CD45RA^+RO^-$/kg and a range of $1\text{-}10 \times 10^6$ $CD3^+$ cells/kg in the sum of the CD45RA-depleted and $CD34^+$ cell products and a minimum of 2.0×10^6 $CD34^+$ cells/kg,.

All fractions will in addition be phenotyped by multi-color flow cytometry for the presence of other cell lineages and T-cell subpopulations (see section 9D3)

c) Identity: Proper identification of all the intermediate and final cell products will be assured by defined process and label controls as specified in standard operating procedures within the cell processing facilities to ensure the recipient is receiving the correct cell products.

d) Stability: The first leukapheresis collection will be stored overnight at 4C prior to CD34 selection and CD45RA depletion, with infusion to take place that same day. Unmanipulated leukapheresis products are routinely stored this duration and longer, particularly in matched unrelated donor transplantation, and we therefore do not anticipate that stability will be a major concern for this study. Viability, which is an important measure of stability, will be assessed immediately after all processing is completed, and infusion will take place within 6 hours after completion and release of the processed products.

APPENDIX C
Positive Culture from Donor Product Action Plan

In the event that a sterility-testing culture sample turns positive, the following actions will take place immediately:

1. The director or designee of the Cell Therapeutics Laboratory will notify the recipient's attending physician and the Principal Investigator or the designated study nurse of the positive culture result.
2. Identification of the organism and sensitivity testing will be completed.
3. The FHCRC PI will notify the FHCRC IRB, the sponsor, Warren Shlomchik, as soon as possible, but no later than 10 calendar days of finding out of the event. The sponsor will notify the FDA as outlined in section 15.
4. After notification of the attending physician, the following actions will take place.
    a. The attending physician will notify the patient (recipient) of the positive culture.
    b. The attending physician or primary care provider will perform a thorough examination of the patient (recipient).
    c. Blood samples will be obtained from the patient (recipient) for cultures (bacterial, fungal, and viral cultures).
    d. Samples of other fluids will be obtained for cultures if clinically indicated.
    e. Assays for identification of the organism and sensitivity testing will be completed and results reported to the attending physician.
5. The patient will be treated with empiric antimicrobial agents until the following endpoints are reached:
    a. Assays for identification and sensitivity of the organism have been completed.
    b. Results of the patient (recipient) blood cultures are available.
    c. The absolute neutrophil count exceeds 500 cells/µl.
    d. Unexplained clinical signs or symptoms of systemic infection have resolved.
    e. Exceptions to the administration of antibiotics must be based upon lack of criteria 5 a-d. These exceptions must be discussed by the attending physician with the PI
6. The patient will be monitored daily for signs of systemic infection.
7. Quality Assurance measures will be enacted.

APPENDIX D
Infusion of Selected Cells

General Guidelines
The selected cells ($CD34^+$ and CD34 depleted CD45RA depleted fraction) will be infused through a blood administration set filter. The cells will be suspended in clinical grade Normosol-R (Hospira) plus HSA (Baxter) for infusion. Central venous access is preferred for infusion. A peripheral IV line may be used only if central access is not available. *Under no circumstances will these cells be irradiated.* The staff caring for the patient must be familiar with the practices and complications of HPC infusion. Staff should be prepared to treat the recipient for an acute hemolytic transfusion reaction.

For FHCRC patients: Questions regarding ABO mismatches should be directed to the SCCA Transfusion Services Office or the SCCA Transfusion Medicine attending on call. Other questions regarding handling of selected cells should be directed to the Medical Director of the Cellular Therapy Laboratory.

For YNHH patients: Questions regarding ABO mismatches should be directed to the Blood Bank Transfusion Medicine Service or the Transfusion Medicine attending on call. Other questions regarding handling of selected cells should be directed to the Medical Director of the Cellular Therapy Laboratory.

Timing
After completion of the conditioning regimen cells should not be infused earlier than 36 hrs after the last dose of chemotherapy, unless specified differently in the protocol or standard treatment plan. Selected cells will be infused as soon as they are available. The $CD34^+$ fraction will be given first followed as soon as possible, by the CD34-depleted CD45RA-depleted fraction. If a delay is anticipated because of timing of conditioning regimen or patient medical status as determined by the attending physician, cells may be stored in the refrigerator (4°C). It is aimed to commence administration of each fraction within 6 hours from the end of processing of that fraction.

Volume
The selected cell volume is generally less than 300 ml total

Pre-medication
At FHCRC no pre-medication will be given unless patient has previously reacted to blood or platelet transfusions, then premedication will be administered as per patient's platelet transfusion guidelines for allergic or febrile reactions. At YNHH diphenhydramine may be given as a premedication as per YNHH standard practice.

Filtration
Product should be filtered through a blood administration set with 150-260 micron mesh size.

Product Infusion Rate
Adults and Pediatrics > 20 kg: Begin at 0.5 times maintenance rate for 15 minutes, then increase to 1.5 times maintenance rate as tolerated.

Monitoring
Vital signs before infusion, after 15 minutes, then hourly during infusion, and at completion of infusion. If any reaction occurs, notify primary care provider.

Concomitant Infusions:
No medications or fluids may be given "piggy-back" with the selected cells, although they may be given through the other lumen of a double lumen catheter. Amphotericin, antibodies, investigational medications or blood products should not be given concomitantly because of difficulty in evaluating reactions. Cells must not be infused during plasmapheresis or dialysis.

Reactions:
Volume Overload: Generally, the $CD34^+$ fraction and CD34 depleted CD45RA depleted cell volumes will be

100ml each so volume overload is not expected. Volume reduction may be required if the cell volume of either individual infusion exceeds 20 ml/kg adult recipient weight, 15 ml/kg pediatric recipient weight, or if the patient is volume overloaded by clinical criteria. Request volume reduction by contacting the Cellular Therapy Laboratory.

Transfusion Reaction: Recipients who have clinically significant antibodies to AB or other antigens found on donor red cells may be at risk for an acute hemolytic transfusion reaction (i.e. major mismatch). Red cell depletion (or recipient plasma exchange) may be indicated in some cases. Patients also may experience hemoglobinuria from damaged cells in the cell inoculum. Delayed hemolysis may occur in the setting of major mismatch and rebound of recipient antibodies after plasmapheresis (if performed). **Refer to the Clinical Coordinator's Patient Information Sheet for instructions regarding red cell reduction of product (or plasma exchange of recipient). The SCCA or YNHH Transfusion Services Offices may also be consulted as a resource for management of specific patients.**

Patients with minor mismatch (i.e. donor antibodies against patient red cells) may experience hemolytic transfusion reactions. Circulating donor antibodies will be removed during processing of the manipulated cells by the Cellular Therapy Laboratory so early hemolytic transfusion reactions are unlikely. Delayed reactions can occur about 5-14 days after marrow infusion. Delayed reactions may occur from formation of antibodies by donor lymphocytes against either recipient or incompatible transfused red cells (see Red Blood Cell Infusion Guidelines).**The SCCA Transfusion Services Office may be consulted as a resource for management of specific patients. For YNH patients, the YUSM Transfusion Services Office may be consulted.**

Allergic Reaction: Recipients may have allergic reactions (chills, fever, hives) to the selected cell product. Please note, these products have been manipulated in the laboratory and may contain foreign proteins or reagents. Treatment is the same (diphenhydramine, meperidine, hydrocortisone) as for reactions to platelet transfusions. For anaphylaxis, treat per Institutional standard practice Guidelines, "Anaphylaxis Emergency / Drug Chart Reference".

Pulmonary Micro-Embolism: Fat and particulates may result in micro-emboli. Patients may complain of chest pain, dyspnea, or coughing. Excessive fat can be removed by centrifugation by the Cellular Therapy Laboratory after discussion with the Medical Director. Slowing of infusion and administration of oxygen may alleviate mild dyspnea during infusion. Excessive fat is highly unlikely in the selected cell products.

Excessive Anti-coagulation: Selected cells will not be anticoagulated with heparin and/or citrate solutions. When heparin is used, rapid or large volume infusions may result in transient anti-coagulation of the recipient.

APPENDIX E
Acute GVHD Staging and Grading Assessment
GRADING OF ACUTE GRAFT-VERSUS-HOST DISEASE[a]

Severity of Individual Organ Involvement		
Skin	+1	a maculopapular eruption involving less than 25% of the body surface
	+2	a maculopapular eruption involving 25-50% of the body surface
	+3	generalized erythroderma involving >50% of the body surface
	+4	generalized erythroderma with bullous formation and often with desquamation
Liver	+1	bilirubin (2.0-2.9 mg/100ml)
	+2	bilirubin (3-5.9mg/100ml)
	+3	bilirubin (6-14.9mg/100ml)
	+4	bilirubin > 15mg/100ml
Gut	Diarrhea is graded +1 to +4 in severity. Nausea and vomiting and/or anorexia caused by GVHD is assigned as +1 in severity. The severity of gut involvement is assigned to the most severe involvement noted. Patients with visible bloody diarrhea are at least stage +2 gut and grade +3 overall	
Diarrhea	+1	≤ 1000 ml of liquid stool/day* (≤ 15ml of stool/kg/day)†
	+2	>1,000 ml of stool/day* (> 15ml of stool/kg/day)†
	+3	>1,500 ml of stool/day* (> 20ml of stool/kg/day)†
	+4	2,000 ml of stool/day* (≥ 25ml of stool/kg/day)†

**In the absence of infectious/medical cause*
†*For pediatric patients*

Severity of GVHD	
Grade I	+1 to +2 skin rash
	No gut or liver involvement
Grade II	+1 to +3 skin rash and/or
	+1 gastrointestinal involvement and/or +1 liver involvement
Grade III	+4 skin involvement and/or
	+2 to +4 gastrointestinal involvement and/or
	+2 to +4 liver involvement with or without a rash
Grade IV	Pattern and severity of GVHD similar to grade 3 with extreme constitutional symptoms or death

a. From "Graft-vs-host disease" Sullivan, Keith M. *Hematopoietic Cell Transplantation* Ed: D. Thomas, K. Blume, S. Forman, Blackwell Sciences; 1999, pages 518-519.

APPENDIX F
GRADING OF CHRONIC GRAFT-VERSUS-HOST DISEASE

Chronic GVHD
Guidelines

Chronic GVHD
Appendix D

APPENDIX G

Potential Adverse Events Associated or Expected with Hematopoietic Cell Transplantation

1. Opportunistic infections, including viral and fungal infections, can results in severe pulmonary, neurologic, hepatic and other organ dysfunction, and possible death.

2. Gastrointestinal toxicity. Nausea and vomiting can be anticipated during the entire course of ablative therapy. Mucositis and diarrhea should be expected. Prednisone can cause GI bleeding.

3. Cardiac toxicity. Cardiotoxicity (for example congestive heart failure, pericardial effusion, EKG changes) is uncommonly associated with chemotherapy agents and TBI and these sequelae may prove lethal.

4. Pulmonary toxicity. Diffuse interstitial pneumonitis of unknown etiology occurs with some regularity after BMT. Interstitial fibrosis and diffuse alveolar hemorrhage occur less frequently. Each are well-described complications of intensive chemotherapy and TBI regimens and may prove lethal.

5. Hepatic toxicity. Veno-occlusive disease of the liver is a common toxicity of high-dose chemoradiotherapy and may result in death. Tacrolimus may cause elevation of ALT/AST.

6. Renal dysfunction. Chemoradiotherapy may uncommonly cause renal dysfunction. More commonly, nephrotoxicity results from tacrolimus and generally responds to dose reduction. Rarely, idiopathic or calcineurin inhibitor-associated hemolytic-uremic syndrome may occur and may be progressive and fatal. A syndrome of moderate renal insufficiency and hemolysis has been seen 5-7 months post HSCT after intensive conditioning plus TBI.

7. Hemorrhagic cystitis, manifested either as gross or microscopic hematuria, is a common toxicity after high-dose chemoradiotherapy, but usually associated with regimens that include cyclophosphamide. Hemorrhagic cystitis may predispose to a long-term increased risk of bladder cancer.

8. Central nervous system toxicity. Radiation and chemotherapy can cause CNS toxicity, including seizures, depressed mental status, or leukoencephalopathy. Calcineurin inhibitors can cause seizures or other CNS toxicity.

9. Marrow aplasia. Severe neutropenia, thrombocytopenia, and anemia, is expected to occur for a period of 7 to 42 days following infusion of marrow. Transfusion of platelets and red blood cells is expected as supportive care. Transfusion of blood products may be associated with acquisition of HIV or a hepatitis virus. Neutropenia may increase the risk for acquiring serious infection. Thrombocytopenia may increase the risk of life-threatening hemorrhage. Hemorrhagic or infectious complications during the expected period of aplasia may result in death.

10. Miscellaneous. Alopecia and sterility are expected complications of the program as a whole. Cataract development is possible after TBI and/or steroids. Deficiencies of growth hormone, thyroid hormone, and sex hormones are possible after TBI. Calcineurin inhibitors can cause transient gingival hyperplasia, tremor, seizure, hypertension, headache, dysesthesia, metabolic complications and hirsutism. Steroid therapy can also contribute to fluid retention, easy bruising, hypertension, aseptic necrosis of bone, metabolic complications including diabetes mellitus and increased susceptibility to infection. Hospitalization during conditioning and recovery period is expected to be 5-9 weeks in duration.

APPENDIX H
Karnofsky Performance Status Scale

KARNOFSKY PERFORMANCE STATUS SCALE

Percentage	
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 signs 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 of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled, hospitalization indicated. Death not imminent
20	Very sick, hospitalization necessary, active supportive treatment necessary
10	Moribund, fatal processes, progressing rapidly
0	Dead

REFERENCE

Karnofsky DA: Meaningful clinical classification of therapeutic responses to anti-cancer drugs. Editorial: Clin Pharmacol Ther 2:709-712, 1961.

APPENDIX I
Weight / Adjusted Body Weight for Drug Dosing

Drug Dosing By Body Size:
Drug dosing will be based on either body surface area (BSA) or body weight.
1. BSA is calculated in M^2. The formula by definition adjusts for both under and over weight individuals.
The formula for this calculation is:

$$\frac{\sqrt{\text{actual weight in kg x height in cm}}}{60}$$

2. Body weight is measured in kg. The ideal body weight (IBW) will be calculated in the following ways

For Adult Patients:
SWOG/CTN Formulas will be used to calculate ideal weights
Males: 50 kg + (2.3 kg/inch over 5 feet)
Females: 45.5 kg + (2.3 kg/inch over 5 feet)
Patients less than 5 feet: subtract 2.3 kg/inch

Height in inches will be rounded to the nearest whole number.

- Height with *inches < ½ inch will be rounded down* to the nearest whole inch

Example: 5 feet 5 ¼ inches will be rounded to 5 feet 5 inches

- Height with *inches > ½ inch will be rounded up* to the nearest whole inch

Example: 5 feet 6 ½ inches will be rounded to 5 feet 7 inches

(Note: The dietitians will document the conversion from height in centimeters to height in inches as well as the rounded height used to calculate the ideal weight on the green Nutrition Demographic / Anthropometric nutrition cardex form.)

For Pediatric Patients:
Post pubertal adolescents (females > 12 years old and males > 14 years old):
Ideal weight will be assessed using the BMI. If the child's normal BMI [weight in kg/(height in meters)2] is between the 25-75th percentile, the child may be considered at IBW.

Children whose BMI exceeds the 75th percentile: Ideal weight will be the 75th percentile BMI weight.
Children whose BMI is below the 25th percentile: Ideal weight will be the 25th percentile BMI weight

Note: When deviating from these age ranges (based on early or late maturity), the dietitian will document the rationale in the nutrition assessment.

Weight / Adjusted Body Weight for Drug Dosing

For both Adult and Pediatric Patients:
Adjusted Body Weight will be calculated as follows:
Ideal Weight + 0.25 (actual weight – ideal weight)

Weight Shifts After Initial Evaluation:
Individuals with significant weight shifts after the initial evaluation will have the adjusted body weight reassessed by the Clinical Nutrition Staff as appropriate.

APPENDIX J
COORDINATING CENTER FUNCTIONS
Outside Center – PI Communication

I. Study Management, data analysis, and Data and Safety Monitoring

a. Study Management:
   i. Each local PI is responsible for selection, training and oversight of local study coordinators
   ii. The Coordinating Center registers subjects on the study and assigns study IDs
   iii. One copy of the research data is retained by the site. Another data set (identified only by study IDs) is transmitted to the Coordinating Center to create the master data file. All data are kept in locked areas and password protected databases accessible only to study staff
   iv. The quality of data is monitored in an ongoing fashion with the study team and corrective action plans instituted as necessary

b. Data Analysis:
   i. Study staff review data for completeness as it is submitted by the sites
   ii. The study statistician is responsible for data cleaning and the conduct of analyses as outlined in the protocol and grant

c. Data Safety and Monitoring:
   i. The collaborating centers will report all adverse events, unanticipated problems and non-compliance as described in section 15.

II. Protocol and informed consent document management

a. A master protocol is maintained by the Coordinating Center and distributed to the sites for customization and local IRB review
b. All protocol and consent modifications initiated by the Coordinating Center are sent to the Collaborating Sites following approval by the Coordinating Center IRB, for review and approval by the local IRB
c. Changes required by local IRBs are reviewed by the Coordinating Center and approved prior to implementation at local sites

III. Assurance of local IRB OHRP-approved assurance

a. Each site provides their OHRP assurance number and evidence of IRB certification
b. Study staff monitor maintenance of institutional assurance and IRB certification

IV. Assurance of local IRB approvals

a. The Coordinating Center maintains copies of the most current collaborating site Consent Forms and IRB approval documentation
b. No site may enroll subjects until the Coordinating Center has received confirmation of local IRB approval
c. Each site is responsible for preparation and submission of their continuing reviews. Any changes to the protocol or consent form will be communicated to the Coordinating Center
d. Sites are required to have active IRB approvals to participate in any study related activities

V. Any substantive modification by the Collaborating Institution related to risks or alternative procedures is appropriately justified

a. The Coordinating Center reviews any modifications to consent forms to ensure that site consents do not delete or change the basic or additional elements or alternatives required in the sample consent form

VI. Informed consent is obtained from each subject in compliance with HHS regulations

a. Subjects must provide written informed consent prior to study participation
b. The Coordinating Center verifies eligibility and signed consent prior to assigning a study ID number

APPENDIX K
Radiotherapy Treatment Guidelines (FHCRC Protocol 2222)

1.1 Total Body Irradiation

1.1.1 Patients
Every patient will receive total body irradiation as part of the preparatory regimen for stem cell transplantation.

The treatment dose regimen will be 165 cGy BID over 4 treatment days to a total dose of 1320 cGy.

Lung shielding will be used as described below.

All male patients having ALL will receive a testicular boost as part of the transplant regimen. 400 cGy for one fraction will be delivered by an en fosse electron field.

1.1.2 Equipment

1.1.2.1 Modality:
High-energy photons with energy ≥ 6MV photons should be utilized. Although there is no upper limit on the energy as long as the skin dose requirements can be met, it is recommended that 18MV or lower be used.

The selection of energy is determined by the dose uniformity criterion.

1.1.3 Target Volume
The total body will be treated, including the head and feet, in one field (except in certain circumstances). Care should be taken to insure that the patient is entirely within the 90% isodose decrement line of the beam (i.e., not in the penumbra region of the beam).

1.1.4 Target Dose
The prescription point is defined as the point along the longitudinal axis of the patient at the midline at the level of the umbilicus (see **Point 5**, section 1.5.4.1). No tissue inhomogeneity correction will be made in the calculation of dose to the prescription point. The absorbed dose along the patient's head to toe axis (line formed by the intersection of the midsagittal plane and the midcoronal plane) shall be within 10% of the prescribed dose. The dose at selected anatomical points shall be calculated and these calculations are to be submitted as part of the quality assurance. Measurements of patient dimensions needed for the calculation of the prescription dose will be made at the time of the simulation for lung blocks. Measurement and calculations of required monitor units necessary for each treatment will be performed in the patient treatment position for AP-PA fields: either upright position or the reclining, lateral decubitus position . In the event the patient is intended to be treated in an upright position, but proves too ill for treatment, dose calculation will have been pre-calculated to permit treatment in the lateral decubitus position).

1.1.4.1 Prescription Point:
The following reference points will be determined:

1. **Head (Point 1)**: this reference point is defined along the longitudinal axis of the skull at the greatest mid-separation (immediately superior to the nasal bridge). The depth should be taken as midway between the entrance and exit points of the opposed radiation beams.
2. **Neck (Point 2)**: this reference point is defined along the patient's longitudinal axis at the level of C3/C4 (approximate mid-neck, but chosen for the thinnest mid-separation of the neck). The point is taken to be midway between the entrance and exit point of the beam.
3. **Upper Mediastinum (Point 3)**: this reference point is defined along the patient's longitudinal axis at the level of the angle of Louis. The reference point is midway between the entrance and the exit points of the opposed beams.
4. **Lower Mediastinum (Point 4)**: this reference point is defined along the patient's longitudinal axis at the level

of the xiphisternal notch. The reference point is midway between the entrance and exit points of the opposed beams

5. **Umbilicus (Point 5)**: THE PRESCRIPTION POINT is defined along the patient's longitudinal axis at the level of the umbilicus. The prescription point is midway between the entrance and exit points of the opposed beams.

6. **Knee (Point 6):** this reference point is defined along the midline in the midplane of the knee at the level of the patella.

7. **Ankle (Point 7)**: this reference point is defined along the midline at the midplane of the ankle at the level of the lateral malleolus.

8. **Shielded Lung Dose (Point 8)**: this reference point is located on the right chest wall under the lung block. It is centered (both medial/lateral and cephalocaudad) under the lung block as projected on the patient's skin. The depth should be taken as midway between the entrance and exit points of the opposed radiation beams. Dose measurements at this location will be taken during a fraction with lung shielding in place.

9. **Unshielded Lung Dose (Point 9)**: This reference point is the same as point 8. Dose measurements at this location will be taken during a fraction without lung shielding in place. The depth should be taken as midway between the entrance and exit points of the opposed radiation beams.

1.1.4.2 Dose definition:
The absorbed dose is specified as centigray (cGy)-to-muscle.

1.1.4.3 Prescribed Dose, and Fractionation, and Timing:
165 cGy will be delivered in one fraction.

1.1.4.4 Dose Rate:
A mid-plane dose rate of between 6 and 15 cGy per minute is required.

1.1.4.5 Dose Uniformity:
The objective is to keep the dose throughout the body, defined to extend to within 2 mm of the skin surface, to at least 90% of the prescription dose. In addition, the brain dose shall not exceed 107% of the prescription dose.

For AP/PA treatments, partial transmission lung blocks will be used to limit the overall total lung dose. The dose at the midpoint of the thickest part of the body, while in the treatment position, should be determined and if necessary, modifications made to the treatment to raise the dose in this region to at least 90% of the prescription dose.

In order to satisfy the requirement that the skin dose at a depth of 2 mm is within at least 90% of the prescription dose, beam spoilers or other equally effective devices should be used. The field size shall be such that no part of the patient extends into the portion of the penumbra region where the dose is less than 90% of the central axis dose.

1.1.5 Treatment Technique
Patients will be treated using AP/PA fields in an upright seated or standing position in a TBI positioning device. Treatment will be delivered with equally weighted parallel opposed portals, with each treatment including both AP and PA fields. An acceptable alternate arrangement will include equally weighted AP-PA parallel opposed fields delivered to the patient in a lateral decubitus position on a treatment couch or gurney.

Young patients requiring anesthesia will be treated in an AP/PA configuration at extended distance. If more than a single field is needed to accomplish treatment, the field junction should be at the level of the thighs.

1.1.5.1 Dose calculation for the Prescription Point
The calculation of the treatment time or the monitor units for the prescribed dose can be carried out using standard techniques. However, TBI presents special problems relative to the routine treatment situation in that the field sizes are much larger and the treatment distances much longer. The TBI percent depth dose (PDD) or Tissue Maximum Ratio (TMR) and output factors should be measured under TBI treatment conditions for a range of phantom sizes to establish the database for TBI beam-on time calculations or to validate the calculation methodology.

Typically, a calculation methodology will be adopted which uses PDD or TMR and output factors measured under standard conditions but then modified to account for the larger treatment distance. For example, modified values for inverse square corrected percentage depth dose or tissue-air ratios and tissue-phantom ratios are necessary for some treatment units when the patient is positioned at a long distance from the photon source and near the floor or one wall of the room. Also, some deviation from an exact inverse square decrease with distance has been demonstrated for certain geometries.

Measurements of dose at the center of a phantom about the size of the typical patient should be performed and compared to the calculated dose. If differences are found, additional correction factors should be introduced to the calculation method.

1.1.5.2 Critical Organ Dose Points
The required dose calculations should be performed for the 9 points referenced above (1.5.4.1). The midline dose at these locations should be recorded on the TBI Summary Form.

1) The dose can be calculated based on the thickness at each location and factors appropriate to the TBI treatment conditions.

It is recommended that entrance and exit TLDs or diodes be placed on the patient at each required dose assessment location. The midline dose can be calculated from these measurements making the appropriate corrections to the readings and then averaging the corrected values.

In younger patients it is also recommended that TLDs or diodes be placed underneath the lung blocks to document the transmission dose and scatter dose.

1.2 Lung Shielding
Lung shielding shall be used in all patients

1.2.1 Lung Block design
Lung blocks will confirm to the following guidelines: The lateral edges will be 1.0 – 1.5 cm from the inner border of the ribs; the inferior edges will be 1.0 – 1.5 cm from the dome of the apex of the diaphragm; the superior borders will be 1.0 – 1.5 cm below the clavicles; the medial border 2.0 – 2.5 cm from the lateral edges of the thoracic vertebral bodies. No contouring of the lung shields will be done around the hilum unless there is a residual abnormal hilar adenopathy, in which case the margins around the hilar mass will be 1.0 – 1.5 cm.

1.2.2 Timing
Lung blocks will be employed for sequential treatments starting with the first treatment. Should patient infirmity preclude upright positioning during a fraction when lung shielding is prescribed, that patient may be treated in the lateral decubitus position without lung shielding, and lung shielding can be deferred until the next treatment fraction. Alternatively, lung blocks may be used in the lateral decubitus position for patients being treated exclusively in that position.

For children receiving TBI under anesthesia, treatments will be performed in a modified supine and prone position, with the appropriate lung shielding as specified in the protocol.

1.2.2.1 Fractionation Schema
For a fractionation scheme of 165 cGy bid x 4 days; lung shielding will be used for the first 4 fractions. This schema is calculated to deliver a nominal dose of approximately 825 cGy to both lungs, without correction for lung homogeneity (660 cGy x 0.25 = 165 cGy + 660 cGy = 825 cGy).

1.2.3 Electron boost
No compensatory electron boost of that portion of the chest wall shielded by the lung blocks is required.

The lung dose will be reported on the TBI Summary form.

1.3 Testes Boost

1.3.1 Patients
All patients with biopsy-proven testicular leukemia at relapse will receive a testicular radiation boost (400 cGy in 1 fraction) prior to TBI. It is recognized that there will some patients who previously received radiotherapy prior to relapse. These patients will be treated similarly.

1.3.2 Equipment
1.3.2.1 Modality
Electron beams. The selection of energy is determined by the dose uniformity criterion, to cover the testes with no less than the 80% isodose curve.

1.3.3 Target Volume
The target volume consists of the testes in the scrotal sac. (Note: Cremasteric reflex may move testes high up in the inguinal canal.)

1.3.4 Target Dose
400 cGy

1.3.4.1 Prescription Point
The prescription point is to Dmax

1.3.4.2 Dose definition
The absorbed dose is specified as centigray (cGy)-to-muscle.

1.3.4.3 Prescribed Dose and Fractionation
The total dose to the prescription point shall be 400 cGy in 1 fraction.

1.3.4.4 Timing
Testicular irradiation should start within 3 treatment days of the beginning of the TBI preparative regimen if they have not already received testicular irradiation as part of their induction therapy.

1.3.4.5 Dose Uniformity:
The variations of dose within the planning target volume shall be within +20%, -20% of the dose to the prescription point. The uniformity requirement can in general be met with an electron beam of appropriate energy provided bolus is used, which is the simplest technique.

1.3.5 Treatment Technique

1.3.5.1 Patient Position
The patient shall be treated in the supine position.

1.3.5.2 Field-shaping
Field shaping can be done with electron cutout blocks of the appropriate thickness.

1.3.6 Normal Tissue Sparing

1.3.6.1 Perineum:
The testes shall be supported posteriorly and, if possible, extended caudally in order to minimize perineal irradiation. The field shall not be angled towards the perineum.

1.3.6.2 Penis:
The penis shall be excluded from the field by fixing it to the skin over the symphysis pubis.

1.4 Questions

Questions regarding the radiotherapy section of this protocol should be directed to the radiation oncology study coordinators, Dr. Kelly or Dr. Roberts:

Ralph Ermoian, MD
Department of Radiation Oncology
1959 NE Pacific St.
Box 356043
Seattle, WA, 98195
Work: 206 598 4100
Email: ralphpe@uw.edu

Kenneth Roberts, MD
Yale University School of Medicine
Department of Therapeutic Radiology
Hunter Radiation Therapy Center
P.O. Box 208040
New Haven, CT 06520-8040
Phone : (203) 785-2957
Fax : (203) 785-4622
Email : kenneth.roberts@yale.edu

1.5 Definitions of Deviation in Protocol Performance

Prescription Dose

Minor Deviation:

The dose to the prescription point differs from that in the protocol by between 6% and 10%.

Major Deviation:

The dose to the prescription point differs from that in the protocol by more than 10%.

Dose Uniformity

Minor Deviation:

The dose to any of the reference points in Section 16.5.5.1 differs from the protocol dose by more than 10% but less than 20%.

Major Deviation:

The dose to any of the reference points in Section 16.5.5.1 differs from the protocol dose by more than 20%.