Protocol Title:

A Phase II Study Evaluating Selective Depletion of CD45RA+ T Cells from Allogeneic Peripheral Blood Stem Cell Grafts from HLA-Matched Related and Unrelated Donors for Prevention of GVHD

NCT Number:

NCT02220985

Document Date:

08/02/2019

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

Current Version: 08/02/2019
Previous Version: 04/16/2019

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2. Introduction

Graft versus host disease (GVHD) is a frequent complication of allogeneic hematopoietic cell transplantation (HCT) from both HLA-matched unrelated or related donors. GVHD-related morbidity and mortality 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 to prevent GVHD following myeloablative allogeneic peripheral blood stem cell (PBSC) transplant from related or unrelated donors, the incidence of grades II – IV acute GVHD is 50-82% and the incidence of extensive chronic graft versus host disease is 41-53%[2-4]. In a large multicenter study that enrolled 550 unrelated donor transplant recipients and randomized them to receive bone marrow or PBSC, 50% of patients receiving either bone marrow or PBSC developed acute GVHD. An increased rate of chronic GVHD was observed amongst recipients of PBSC (53%, CI 45 to 61) compared with recipients of bone marrow (41%, CI 34 to 48). At two years after HCT, 57% of recipients of PBSC and 37% of recipients of bone marrow still required systemic immunosuppression to manage GVHD. There was no significant difference in overall survival between the groups and the rate of graft failure was lower amongst PBSC recipients (3% CI 1-5%) compared to bone marrow recipients (9% 6-13%)[2]. Despite the increased risk of cGVHD with T cell-replete PBSC grafts in unrelated donor (URD) HCT, the use of PBSC for URD HCT has been increasing substantially, and represented 76% of URD HCTs performed in 2011[2,5]. There is a clear need to develop strategies to reduce the rate of GHVD, particularly in PBSC transplants. An optimal approach would preserve the benefit of rapid engraftment and low rate of graft failure associated with the use of PBSC, but substantially reduce the rates of acute and chronic GVHD.

In HCT using HLA-matched related or unrelated donors, GVHD results from donor T cell recognition of minor histocompatibility (H) antigens expressed on recipient tissues[6]. 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 in some studies has been associated with an increased risk of graft rejection and leukemia relapse[7,8]. Laboratory studies showed that donor T cells specific for recipient minor H antigens are found predominantly within the naïve subset of $CD8^+$ T cells, identified by their expression of 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 [9,10].

We conducted a first-in-human Phase II trial at FHCRC and Yale University School of Medicine (YUSM) in which patients with high-risk leukemia received myeloablative conditioning and HCT from an HLA identical donor with PBSC that were depleted of $CD45RA^+$ T_N by immunomagnetic selection. The outcomes of the first 30 patients treated on the study are outlined in detail in Section 3H. Briefly, steroid-refractory acute GVHD (aGVHD) did not occur and severe aGVHD was rare (7%, 95% confidence interval [CI] 0-16%). Chronic GVHD was very infrequent (12%, CI 0-26%) and no patient developed sclerodermatous GVHD or fasciitis. Lymphocyte recovery was comparable to allogeneic T-cell-replete HCT and functional virus-specific T-cells were transferred from donor to recipient. This protocol will extend our studies of selective T_N depletion to include recipients of both HLA-matched related (MRD) and unrelated donor (MUD) HCT, and evaluate the T_N-

depletion approach in patients who require a lower-intensity conditioning regimen. We will also evaluate whether the addition of methotrexate (MTX) (high-intensity conditioning recipients) or MMF (lower-intensity conditioning recipients) will further reduce the risk of acute GVHD in recipients of T_N-depleted HCT.

3. Background

3A. GVHD

The pathogenesis of GVHD involves multiple interacting factors including tissue damage resulting from the conditioning regimen, the activation and proliferation of mature donor αβ T cell receptor (TCR) expressing T cells that recognize recipient alloantigens presented as peptides by class I and II major histocompatibility complex (MHC or Human Leukocyte Antigens, HLA in humans) molecules on antigen presenting cells (APCs), their release and induction of inflammatory cytokines, and finally tissue infiltration wherein alloreactive T cells cause tissue damage [1,11]. The mature T cell repertoire is "tolerant" to peptides derived from self-proteins due to thymic deletion and/or peripheral tolerance mechanisms [12]. 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 gene polymorphisms, and those peptides can be recognized as minor H antigens by donor T cells [6]. The tissues that are most frequently damaged during acute GVHD are the skin, gastrointestinal tract, and liver, although other organs can be involved, particularly when acute GVHD evolves into chronic GVHD. Chronic GVHD has a characteristic clinical presentation that often resembles autoimmune diseases and is distinct from acute GVHD. Chronic GVHD can be highly debilitating and prolonged and has a 20-50% mortality rate due to immune dysregulation and opportunistic infections [13].

The administration of immunosuppressive drugs that interfere with T cell activation or proliferation such as methotrexate (MTX), cyclosporine, FK506, sirolimus, and mycophenolate mofetil, alone or in various combinations, is used at most centers to prevent GVHD [14]. The development in the early 1980s of the combination of a short course of MTX 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 [14,15]. With the use of a calcineurin inhibitor and MTX following myeloablative BMT or PBSCT from unrelated donors the incidence of grade II – IV acute GVHD is 50-82% and the incidence of extensive chronic GVHD is 41-53% at the FHCRC and other centers. The frequency of acute and chronic GVHD after MRD donor HCT is only slightly lower [2-4].

Patients who develop GVHD typically require treatment with additional immunosuppressive drugs, which increases their risk of post-transplant infections and may diminish the graft versus leukemia (GVL) effect [16]. 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 directed to malignant cells.

3B. T Cell Depletion to Prevent GVHD

Many centers have used partial or complete depletion of T cells from the donor bone marrow or PBSC grafts as an alternative approach to pharmacologic immunosuppression for preventing GVHD [7,8,17-21]. A variety of methods have been used to remove T cells including soybean lectin agglutination, monoclonal antibodies, and positive selection of

$CD34^+$ hematopoietic progenitors [7,8,17-22]. 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 minimum threshold dose of donor T cells needed for development of 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 [7,8,17-21] but recipients of T cell-depleted grafts have poor reconstitution of T cell immunity to pathogens, and some studies found T-cell depletion to be associated with an increased risk of graft rejection and relapse [7,8,17,23,24]. The Bone Marrow Transplant Clinical Trials Network published a multicenter trial of complete T cell depletion for AML patients undergoing HCT from HLA-identical sibling donors [20,22,24]. The BMT-CTN study 0303 used TBI (13.75 Gy), Cyclophosphamide, and ATG 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 provided a 4.9 log_{10} removal of total T cells (3.2-5.9), including both the memory and naïve subsets. All patients engrafted with secondary graft failure occurring in only 1 of 44 patients. Low rates of acute GVHD (grade II-IV 22.7%, III-IV 4.5%) and extensive chronic GVHD (6.8%) were observed. The DFS and OS at three years in all patients that received $CD34^+$ selected transplants were 53% and 56% respectively; 58% and 60% for patients transplanted in CR1; and 29% and 38% for patients transplanted in CR2.[20,22] The OS and DFS figures are comparable to those seen after T-replete transplant, at least for patients in CR1 [3,24-27]. However, as expected $CD34^+$ selected transplant recipients experienced a high risk of opportunistic viral infections (57% of patients). In particular, high-level EBV reactivation requiring treatment was observed in 18% of patients and 1 of 44 patients died of EBV-PTLD [24].

Two studies at Memorial Sloan Kettering Cancer Center employed a similar conditioning regimen of TBI (13.75 Gy), thiotepa (10 mg/kg over two days), fludarabine (125 mg/m^2 over five days) and (in URD recipients only) equine ATG (60 mg/kg) and performed $CD34^+$ selection of stem cells from G-PBSC (N=29) or BM (N=6) from donors with the Isolex Device followed by sRBC-rosette depletion of T cells. This approach achieved a 5-log depletion of T cells [18,19]. Amongst MRD recipients, acute GVHD was limited to grade II and was seen in 8% of patients, extensive chronic GVHD was reported in 9% of patients, and DFS at 3 years was 61%. Amongst MUD recipients, the rates of aGVHD II-IV and extensive chronic GVHD were reported to be 5.8% and 11% respectively. DFS 4 years was 57 %, whilst the relapse rate was surprisingly low at 6% at 4 years. Opportunistic infections were also frequent in these trials. Serious adenoviral infections occurred in 14.7% of patients including one death and EBV reactivation occurred in 9% of patients including one fatal EBV PTLD.

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

Graft manipulations that selectively deplete T cells that recognize minor H antigens and retain T cells specific for pathogens could reduce GVHD and improve reconstitution of T cell immunity. 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, T cell receptor diversity, prior exposure to cognate antigen, and functional activity [28,29]. In mice, the T_N subset is $CD44^-$ $CD62L^+$, and the T_M subset is $CD44^+$ and can be further subdivided into

$CD62L^+$ central memory (T_{CM}), and $CD62L^-$ effector memory (T_{EM}) populations [29]. 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 histocompatibility differences. The first study to examine this question employed a multiple minor H antigen mismatched $CD4^+$ dependent GVHD model [B10.D2 (H-2^d) → BALB/c (H-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 [30].

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 [30-38][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 [31]. Similar results were obtained in an MHC-mismatched model. Other groups have reported that T_{CM} do not cause GVHD at all [33,37]. These results demonstrate that in murine allogeneic minor H antigen and MHC mismatched bone marrow transplantation, T_N are potent inducers of GVHD whereas T_M exhibit limited capacity to cause GVHD.

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[30]	$CD4^+$ $CD44^+$ $CD62L^-$ (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[31]	$CD8^+$ $CD44^+$(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. J. Immunology 182; 5938-5948 2009 [36]	$CD8^+CD44^+CD62L^+$(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: 1534-1541, 2004[32]	$CD4^+$ + $CD8^+$ $CD62L^-$ (T_{EM}) vs $CD4^+$ + $CD8^+$ $CD62L^+$ (T_N & 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	Chen B et al Blood 109: 3115-3123 2007[33]	$CD4^+$&$CD8^+CD62L^+$ $CD44^-$(T_N) vs. $CD4^+$&$CD8^+$ T_M (all other), $CD4^+T_M$ (all other), $CD4^+$&$CD8^+$ $CD62L^+$ $CD44^-T_{CM}$	C57BL/6 (H-2 b) →BALB/c (H-2d) And C57BL/6 (H-2 b) to BALB/c (H-2d)	T_N Severe GVHD T_M total No GVHD $CD4^+T_M$No GVHD T_{CM} No GVHD
MHC Mismatched	Dutt S et al J. Immunology 179: 6547-6554	$CD4^+CD62L^+$ $CD44^-$(T_N) $CD4^+CD62L^-CD44^+$(T_{EM})primed $CD4^+CD62L^-CD44^+$(T_{EM})unprimed	C57BL/6 (H-2 b) →BALB/c (H-2d	T_N Severe GVHD T_{EMP} Severe GVHD T_{EMUP} No/min

	2007 [34]			GVHD
MHC Mismatched	Zheng et al, Blood, 111: 2476-2484.2008 [35]	$CD4^+CD44^+CD62L^-(T_{EM})$ vs CD4+CD44-CD62L-(T_N)	$B6^{bm12}$→B6	No GVHD in T_{EM} recipients; GVHD in T_N recipients.
MHC Mismatched	Zheng H et al. J. Immunology 182; 5938-5948 2009 [36]	$CD8^+CD44^+CD62L^+(T_{CM})$ vs $CD8^+$ $CD44^-$ $CD62L^+(T_N)$	C57BL/6 (H-2 b) →BALB/c (H-2d)	T_N Severe GVHD +GVL T_{CM} Milder GVHD +GVL
MHC Mismatched	Dutt S et al, Blood 117, 3230-3239 2011 [37]	$CD8^+CD44^-$ (T_N) vs $CD8^+CD44^+(T_M)$	C57BL/6 (H-2 b) to BALB/c (H-2d)	T_N Severe GVHD +GVL T_M minimal GVHD+GVL
MHC Mismatched (Rat Model)	Xystrakis E et al. Eur J Immunol, 34: 408, 2004 [38]	$CD4^+$ $CD45RC^{high}$ (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

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

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 greater T cell receptor (TCR) diversity than T_M [39,40]. 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 but in humans does not appear to cause GHVD [41,42], and cross reactivity with minor H antigens is likely to be 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 significantly higher in the T_N subset than in the T_M subset. Indeed, only very rare cells (~1/2,000,000-1/6,000,000) 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 [10]. Others have subsequently demonstrated greater responses to minor H antigens in vitro by human $CD4^+$ T_N compared with $CD4^+$ T_{CM} or T_{EM}. [43]

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 suggested that selective depletion of T_N from human PBSC grafts is a rational strategy to investigate for reducing GVHD. The CD45RA molecule is expressed stably on the surface of all $CD8^+$ and $CD4^+$ T_N in G-CSF mobilized PBSC but a minor subset of $CD34^+$ cells that contain hematopoietic progenitors also express CD45RA. Thus, to preserve $CD34^+$ progenitors, we used sequential positive selection of $CD34^+$ cells

using Miltenyi CD34 immunomagnetic selection, followed by depletion of $CD45RA^+$ cells from the CD34-negative fraction. The two-step approach also allowed for a defined T cell dose without compromising the number of $CD34^+$ cells infused.

A GMP-grade murine αCD45RA monoclonal antibody that is directly conjugated to Miltenyi iron dextran beads was produced by Miltenyi Biotec under contract from the NIH RAID program. Using the new αCD45RA mAb-conjugated bead and the existing αCD34-conjugated bead we have now successfully completed more than 40 clinical scale cell selection procedures, including preclinical validation runs and procedures for 33patients who have been treated on our first Phase II Study of Selective Depletion of $CD45RA^+$ T Cells from Allogeneic Peripheral Blood Stem Cell Grafts for the Prevention of GVHD using HLA-matched related donors (see section 3H).

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

We evaluated CD45RA depleted hematopoietic cell products for retention of functional memory T cells specific for common pathogens including CMV, EBV, influenza, parainfluenza, HSV and VZV. For persistent viruses such as CMV and EBV, a portion 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, EBV and adenovirus in PBSC before and after depletion of $CD45RA^+$ cells using an interferon gamma Elispot assay. Interferon-producing virus-specific $CD8^+$ T-cells were present in both the CD45RA-depleted fraction and unselected PBSC at comparable frequencies. $CD4^+$ 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, HSV, VZV, Adenovirus, Influenza and Parainfluenza antigens before and after depletion of $CD45RA^+$ T-cells from G-PBSC products and found that $CD4^+$ T_M responses to all the antigen preparations were present in CD45RA depleted PBSC. These results demonstrate that $CD8^+$ and $CD4^+$ memory T cells to common opportunistic pathogens are retained after depletion of $CD45RA^+$ cells [9].

3H. <u>Preliminary analysis of the first-in-human study to evaluate selective depletion of naïve T cells from PBSC grafts in patients with acute leukemia or MDS undergoing allogeneic HLA-identical HCT from a related donor.</u>

We are completing a phase II study to evaluate selective depletion of naïve T cells from PBSC grafts in patients with acute leukemia or MDS undergoing allogeneic HLA-identical HCT from a related donor. We employ a myeloablative preparative regimen consisting of fludarabine, thiotepa, and TBI. The product that is administered to each patient consists of infusions of purified $CD34^+$ cells, obtained by Miltenyi CliniMACS selection of $CD34^+$ cells from G-CSF mobilized apheresis products, and $CD45RA^-$ cells obtained by depletion of $CD45RA^+$ cells from the flow-through remaining after the $CD34^+$ cell selection [9].

The goals in this trial is to administer a $CD34^+$ cell dose of $>2.0 \times 10^6$/kg of recipient body weight and a total T cell dose of 10×10^6 $CD3^+$ cells/kg, of which $<7.5 \times 10^4$/kg are $CD45RA^+$ $CD45RO^-$ naïve T cells were readily achieved in each of the first 33 patients. All patients received $>5.0 \times 10^6$ $CD34^+$cells/kg, with the median $CD34^+$ dose being 7.6×10^6 cells/kg. The median $CD3^+$ T cell dose administered was 10.0×10^6 cells/kg (range 9.9-10.0). All patients received $<7.5 \times 10^4$ $CD45RA^+$ RO^- naïve T cells/kg, the median dose was 0.36×10^4 $CD45RA^+$ RO^- naïve T cells/kg (range 0.05-7.46). Approximately two thirds of the residual

$CD45RA^+$ RO^- naïve T cells were within the $CD34^+$ cell fraction and one third were from the $CD45RA^-$depleted PBSC product. In summary, our procedure to prepare a stem cell graft that contains $CD34^+$ stem cells and memory T cells, and is depleted of naïve T cells is effective and reproducible [9].

We have performed an interim analysis of the first 30 patients treated on the trial, including patients with ALL (N=17), AML (N=8), MDS (N=3), mixed linage leukemia (N=1), CML (N=1) with an age range of 19-55 years. Thirteen patients were in CR1 without minimal residual disease (MRD) prior to HCT, seven patients were in first morphological remission but had MRD, and five patients were in CR2 or 3 without MRD, three patients were in CR2 or 3 with MRD and two patients had refractory disease with >5% bone marrow blasts. The median time of follow-up for surviving patients is 592 days (100-1432) (December 2013). The preliminary analysis of engraftment, non-relapse mortality, overall and disease free survival is outlined in Table 2, and is encouraging.

Table 2: Engraftment and survival amongst first 30 patients treated with naïve T cell-depleted PBSC (FHCRC Protocol 2222)

Outcome	
Neutrophil engraftment (>500/μl)	30/30 patients
Time to neutrophil engraftment	Median 13 days (range 9-18 days)
Overall survival 2 years (95% confidence interval)	80% (64-96%)
Disease free survival 2 years (95% confidence interval)	68% (49-86%)
Relapse 2 years (95% confidence interval)	20% (4-36%)
Non-relapse mortality (95% confidence interval)	12% (0-24%
Non-relapse mortality patients <40years	0/16
Non-relapse mortality all patients ≥40 years	3/14

a. Acute GVHD: Acute GVHD grade II-III that was universally steroid-responsive, has been observed in 20/30 (67%, 95% CI 50-84%),) of patients. Eighteen patients developed grade II GVHD and responded rapidly to corticosteroids. Two other patients were considered to have grade III GVHD on the basis of large volume diarrhea but also responded rapidly to prednisone with no subsequent recurrence. In each of the 20 cases of grade II-III GVHD, GVHD predominantly involved the upper GI. Skin involvement was also seen in 7/20 patients while liver GVHD has not been observed to date. A biopsy of the intestinal tract was obtained in all patients diagnosed with gastrointestinal GHVD. The histopathologic changes were considered minimal or mild, consistent with the rapid response to prednisone. The rate of mild upper gastrointestinal GVHD is similar to the historical experience for adult patients treated with myeloablative TBI containing conditioning with T cell replete BMT or PBSCT at FHCRC where there is a high diagnostic sensitivity for mild gastrointestinal GVHD without skin involvement [44].

b. Chronic GVHD: Thus far 3 (12%, 95% confidence interval 0-26%) recipients of T_N-depleted HCT have been diagnosed with cGVHD meeting NIH consensus criteria.. The frequency of chronic GVHD appears to be considerably lower than the historical experience of 45-50% for adult patients receiving unmanipulated PBSC transplants from HLA-identical sibling donors[27].

c. Discontinuation of immune suppression: Of the 22 patients who are alive ≥ 6 months after HCT and have not relapsed, 19 have completed all IS. The rate of completion of prednisone and all IS appears to be substantially quicker amongst recipients of T_N-

depleted HCT compared to recipients of T cell-replete PBSCT treated at FHCRC and other centers [2].

d. Immune reconstitution: The median $CD3^+$ T cell numbers /mm^3 of peripheral blood in recipients of naïve T cell depleted transplant was above 300 at 28 days after transplant and remained at this level for the first three months before increasing to 800/mm^3 at 1 year post transplant. $CD4^+$ T cells median values are generally above 50 $CD4^+$ T cells/mm^3 of blood for the first 3 months and above 200/mm^3 by one year. The numbers of $CD4^+CD3^+$ and $CD4^+CD8^+$ T cells in peripheral blood after naïve T cell depleted HCT exceed those seen after TCD HCT over the first 12 months after HCT.[20] EBV reactivation has been monitored weekly by quantitative PCR and there has been only one very low level reactivation in a single measurement (41 copies). In comparison, a much higher 18% rate of EBV reactivation was seen after TCD HCT.[19,20] In summary, in the first year after HCT, overall immune reconstitution appears to be superior in recipients of naïve T cell depleted grafts compared to total TCD transplants, with a corresponding reduced level of viral reactivation.

3I. Proposed clinical trial of naïve T cell depletion for MUD and MRD HCT recipients

The data from the initial clinical trial suggests that naïve T cell depletion of PBSC markedly reduces the frequency, severity and duration of chronic GVHD relative to unmanipulated PBSC, and improves immune reconstitution relative to TCD HCT. The frequency of acute GVHD is not reduced using naïve T cell depletion and tacrolimus monotherapy compared to T cell replete HCT with conventional calcineurin inhibitor and MTX prophylaxis, but the acute GVHD observed is mild and steroid responsive. The mechanism of the predominantly gastrointestinal GVHD syndrome observed after naïve T cell depleted HCT is the subject of ongoing research and may involve activation of T_M by microbial or self-antigens in a gastrointestinal tract that has been damaged by intensive chemoradiotherapy and resulted in high levels of pro-inflammatory cytokines present after HCT. The development of an acute GHVD syndrome in this context may not necessarily require the presence of alloreactive T cells, which may explain the success in rapidly eliminating all immunosuppression.

While these results are exciting and support the overall hypothesis that depleting T_N can reduce serious GVHD, we see four major issues that should be addressed with continued clinical research. First, our initial results are with only 30 patients, and a subsequent trial involving more patients is necessary to substantiate the current conclusions on important secondary endpoints, including chronic GVHD. Second, less than 50% of transplant candidates have a MRD. Therefore to maximize the clinical impact of naïve T cell depletion, this approach needs to be evaluated in MUD graft recipients. Third, the intensity of the conditioning regimen required us (guided by the FDA) to restrict the protocol to patients ≤55 years of age, whereas acute leukemia and RAEB often affect older patients. Fourth, while the aGVHD we observed was mild and 100% steroid responsive, we seek to test whether the addition of a second immunosuppressive agent can decrease aGVHD without compromising engraftment, immune reconstitution and leukemia remissions.

To address each of these issues we propose a second clinical trial of naïve T cell depletion in MRD and MUD HCT recipients that will stratify patients into 4 subgroups. Arms A (MRD) and C (MUD) will be with the same TBI, thiotepa, fludarabine

conditioning regimen as in the initial trial (high-intensity myeloablative conditioning), with the addition of MTX to tacrolimus, which was previously given as monotherapy. Arms B (MRD) and D (MUD) will evaluate naïve T cell depletion with a lower intensity myeloablative conditioning regimen, and use tacrolimus and MMF as GVHD prophylaxis. The lower intensity regimen consisting of cyclophosphamide, fludarabine, thiotepa and 400cGy of TBI without serotherapy has been used successfully in cord blood transplantation with a high rate of engraftment (97% neutrophil engraftment), and acceptable risks of non-relapse mortality (20% day 180) and leukemic relapse (11% 2 years).[45] Patients will be enrolled at both the FHCRC and University of Pittsburgh Medical Center (UPMC) with the FHCRC serving as the coordinating site.

4. Objectives

4A. Primary objectives

1. To estimate the probability of developing chronic GHVD among patients who receive T_N-depleted PBSCT in each of the following groups:
   a) Arm A: Patients who receive T_N-depleted PBSC from a MRD following high-intensity myeloablative conditioning (TBI 1320cGy, Thiotepa, Fludarabine) and pharmacological immunosuppression with tacrolimus and methotrexate.
   b) Arm B: Patients who receive T_N-depleted PBSC from a MRD following lower-intensity myeloablative conditioning (TBI 400cGy, Thiotepa, Fludarabine and Cyclophosphamide) and pharmacological immunosuppression with tacrolimus and mycophenolate mofetil (MMF).
   c) Arm C: Patients who receive T_N-depleted PBSC from a MUD following high-intensity myeloablative conditioning (TBI 1320cGy, Thiotepa, Fludarabine) and pharmacological immunosuppression with tacrolimus and methotrexate
   d) Arm D: Patients who receive T_N-depleted PBSC from a MUD following lower-intensity myeloablative conditioning (TBI 400cGy, Thiotepa, Fludarabine and Cyclophosphamide) and pharmacological immunosuppression with tacrolimus and MMF.

2. Acute GVHD
   a. To estimate the probability of aGVHD grade II-IV following T_N-depleted (T_ND) PBSCT with tacrolimus and methotrexate (Arm A) or MMF (Arm B) GVHD prophylaxis in MRD recipients. The probability in Arm A will be compared to the rate of aGVHD grade II-IV (67%) observed after T_ND PBSCT and tacrolimus alone in patients treated on FHCRC protocol 2222.
   b. Estimate the rate of aGVHD grade II-IV following T_ND PBSCT with tacrolimus and methotrexate (Arm C) or MMF (Arm D) prophylaxis in recipients of MUD HCT.

3. Estimate the probability of graft failure in recipients of $CD45RA^+$ T_N-depleted PBSCT with tacrolimus and MTX or MMF GVHD prophylaxis.

4B. Secondary objectives

In recipients of $CD45RA^+$ T_N-depleted PBSC in each of the four arms:

1. Estimate the probability of transplant-related mortality by day 100
2. Estimate the probability of relapse
3. Evaluate immune reconstitution and pathogen-specific immune reconstitution

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 (Arms A or C only)
   d. Acute myeloid leukemia in relapse or primary refractory disease with a circulating blast count of no more than 10,000/mm^3 (Arms A or C only)
   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 or related neoplasm (including but not limited to 'biphenotypic', 'undifferentiated' or 'ambiguous lineage' acute leukemia, blastic plasmacytoid dendritic cell neoplasm or lymphoblastic lymphoma)
2. Patient age 0-60 years old at time of enrollment
   a. Patients 0-49 years old will be enrolled in Arm A or C (high-intensity)
   b. Patients 50-60 years old will be enrolled in Arm B or D (lower intensity). Patients eligible for Arms B or D also include those who have received previous allogeneic HCT, or who have co-morbid conditions rendering them unsuitable for high-dose conditioning, determined in consultation with the principal investigator.
3. Patient with a HLA-matched (HLA-A, B, C, and DRB1 molecularly matched) unrelated donor or related donor capable of donating PBSC.

5B. Exclusions

1. Patients with CNS involvement refractory to intrathecal chemotherapy and/or standard cranial-spinal radiation
2. Patients on other experimental protocols for prevention of acute GVHD
3. Patient weight ≥100kg. Patients ≥70kg with MUDs must be discussed with the principal investigator.
4. Patients who are HIV+
5. Patients with uncontrolled infections for whom myeloablative HCT is considered contraindicated by the consulting infectious disease physician (Upper respiratory tract viral infection does not constitute an uncontrolled infection in this context)
6. Patients with organ dysfunction.
   Patients will be excluded from Arm A or C if they have:
   a. Renal insufficiency (creatinine >1.5 mg/dl) at the present time. Patients with a known history of creatinine >1.5 mg/dl must have a current estimated creatinine clearance of >40ml/min.
   b. Cardiac ejection fraction < 45%
   c. DLCO corrected < 60%. Patients who are unable to perform pulmonary function tests (for example, due to young age and/or developmental status) will be excluded if the O2 saturation is < 92% on room air.
   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 the high-intensity arms of

protocol is contraindicated for that patient the patient may be considered for treatment on the lower intensity arm of the protocol or 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 high-intensity arms of the protocol.

Patients will be excluded from Arm B or D if they have

a. Renal insufficiency (Creatinine >2.0mg/dl) at the present time. Patients with a known history of creatinine > 1.5 mg/dl must have a current estimated creatinine clearance > 40 ml/min.
b. Cardiac ejection fraction <35%
c. DLCO corrected <50% Patients who are unable to perform pulmonary function tests (for example, due to young age and/or developmental status) will be excluded if the O2 saturation is < 92% on room air. Patients with a DLCO 50-60% must also have a pO2 of >80mmHg.
d. Liver function abnormality. Patients who have LFTs ≥ 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). Patients with fulminant liver failure, cirrhosis with evidence of portal hypertension or bridging fibrosis, alcoholic hepatitis, esophageal varices, a history of bleeding esophageal varices, hepatic encephalopathy, or correctable hepatic synthetic dysfunction evidenced by prolongation of the prothrombin time, ascites related to portal hypertension, bacterial or fungal abscess, biliary obstruction, chronic viral hepatitis with total serum bilirubin > 3mg/dL, and symptomatic biliary disease will be excluded.

7. Patients will be excluded from Arms A and C if they have received a previous myeloablative transplant. Patients who have received a prior HCT at least 6 months prior may be considered for inclusion on Arms B or D after discussion with the PI.
8. Patients with a life expectancy <3 months from co-existing disease other than the leukemia or RAEB
9. Patients who are pregnant or breast-feeding
10. Fertile patients of child bearing age unwilling to use contraception during and for 12 months post-transplant
11. Patients with a significant other medical conditions that would make them unsuitable for transplant
12. Patients with a known hypersensitivity to tacrolimus, methotrexate (Arm A or C) or MMF (Arm B or D).

6. Donor Selection

6A. Inclusions

1. HLA-matched related donors ≥18 years and capable and willing to donate PBSC (Arms A and B)
2. HLA-matched unrelated donors (HLA-A, B, C, and DRB1 matched based on high-resolution typing) capable and willing to donate PBSC (Arms C and D)

6B. Exclusions

1. Donors who are HIV-1, HIV-2, HTLV-1, HTLV-2 seropositive or with active hepatitis B or hepatitis C virus infection
2. 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)(iii) (urgent medical need) or with 21 CFR 1271.65(b)(i) (allogeneic

use in a first-degree or second-degree relative)
3. Unrelated donors donating outside of the USA.

7. Evaluation and Counseling of Patient

Patients will be referred to the Fred Hutchinson Cancer Research Center (FHCRC) or University of Pittsburgh Medical Cancer (UPMC) for consideration of a hematopoietic cell transplant. The protocol will be discussed thoroughly with patient, and other family members if appropriate, and all known risks to the patient 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 will be obtained using forms approved by the FHCRC Institutional Review Board (IRB) or the University of Pittsburgh IRB. A conference summary detailing what was covered will be dictated for the medical record.

Related donors will be evaluated separately at the FHCRC or UPMC. The protocol will be discussed thoroughly with donor (and other family members if appropriate). The procedure and alternative forms of therapy will be presented as objectively as possible, and the known risks and hazards of the procedure explained to the donor and in the case of minors, to the donor's responsible legal guardian. Consent will be obtained using forms approved by the FHCRC IRB or the University of Pittsburgh IRB.

Unrelated donors will be managed by the NMDP (US donors) or DKMS (German donors).

8. Protocol Registration

8A. Overall Procedures:
Patients enrolled at FHCRC will be assigned to the protocol by the SCCA (Seattle Cancer Care Alliance) Intake Coordinator. Once full consent has been obtained the FHCRC 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 P2684 (**Protocol Patient Performance Program** (PPPP) database by FHCRC study staff.

8B. UPMC Procedures:
When a patient at UPMC is thought to be eligible, consent will be obtained from the (related) donor and the recipient. When signed consents are obtained, UPMC study staff will notify the FHCRC study research nurse by email and provide copies of the signed consent forms (consent for patient and prescreening 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 UPMC research nurse within 48 business hours after receipt of the signed patient consent and (related) donor prescreening consent forms.

9. Plan of Treatment

9A. Conditioning Regimens

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.

Arms A and C (High-intensity myeloablative conditioning): 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 Tables 3 and 4.

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.
3. Patients will receive fludarabine 25 mg/m^2/day (m^2 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. Treatment Plan Arm A (High-intensity conditioning, MRD)

Day of the week (*example only*)	**M**	**T**	**W**	**Th**	**F**	**S**	**S**	**M**	**T**	**W**	**Th**	**F**	**S**	**S**
Day relative to transplant of CD34$^+$ cells	**-10**	**-9**	**-8**	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**0/1**	**2**	**3**
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 CD34$^+$ stem cells and CD45RA$^+$-depleted cells											X	+/-[1]	+/-[2]	+/-[2]
Donor GCSF						X	X	X	X	X	+/-			
Donor apheresis										X	+/-[1]	+/-[2]	+/-[2]	
Cell selection											X	+/-[1]		

1 An apheresis collection will be performed on day -1, and may also be performed on day 0. The selected product will be infused on day 0. (Also see section 9C, 9D &9E and Appendix A). 2On rare occasions an additional collection of PBSC, or bone marrow could be required.

Table 4. Treatment Plan Arm C (High-intensity conditioning, MUD)

Day of the week (*example only*)	**M**	**T**	**W**	**Th**	**F**	**S**	**S**	**M**	**T**	**W**	**Th**	**F**	**S**	**S**
Day relative to transplant of CD34$^+$ cells	**-10**	**-9**	**-8**	**-7**	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**0/1**	**2**	**3**
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 CD34$^+$ stem cells and CD45RA$^+$-depleted cells											X	+/-[2]	+/-[2]	+/-[2]

Donor GCSF						X	X	X	X	X	+/-	+/-		
Donor apheresis										X	+/-[2]	+/-[2]	+/-[2]	
Cell selection											X	+/-[2]		

[1] An apheresis will be collected on day -1, and transported as soon as possible on day-1. The selected product will be infused on day 0. [2] On infrequent occasions a second apheresis could be required to make the cell selection goals and would be collected on day+0, +1 or +2 and infused on day+1, +2 or +3. (Also see section 9C, 9D &9E and Appendix A).

Arms B and D (Lower-intensity myeloablative conditioning): The conditioning regimen will consist of cyclophosphamide, fludarabine, thiotepa, and fractionated total body irradiation (TBI) and will be administered as outlined below and shown schematically in Table 5 and 6.

1. Cyclophosphamide 50 mg/kg (adjusted body weight - see appendix I) will be given on day -6 administered intravenously over 1 hour. Mesna at a dose of 100% of the cyclophosphamide dose in four divided doses will be administered according to institutional standard practice. Patients will receive intravenous hydration for at least 8-10 hours (for example 2 hours preceding and 8 hours following cyclophosphamide) according to institutional standard practice.
2. Patients will receive fludarabine 30 mg/m^2/day (m^2 based on actual weight; see appendix I) administered intravenously over approximately 30 minutes for 5 days beginning on the first day of cyclophosphamide (days -6 to -2).
3. 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 -5 and -4). 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).
4. TBI will be given as 200 cGy fractions once per day x 2 days (days -2, -1). Total dose 400cGy.
5. GCSF-mobilized CD34-enriched PBSC and CD45RA-depleted cells will be infused on day 0.

Table 5. Treatment Plan Arm B (Lower-intensity conditioning, MRD)

Day of the week (*example one*)	**F**	**S**	**S**	**M**	**T**	**W**	**Th**	**F**	**S**	**S**
Day of the week (*example two)*	**T**	**F**	**S**	**S**	**M**	**T**	**W**	**Th**	**F**	**S**
Day relative to transplant of CD34$^+$ cells	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**0/1**	**2**	**3**
Cyclophosphamide 50mg/kg x 1 day	X									
Thiotepa 5 mg/kg/day x 2 days		X	X							
Fludarabine 30 mg/m^2/day x5 days	X	X	X	X	X					
TBI 200cGy x 2 days					X	X				
Infusion of CD34$^+$ stem cells and CD45RA$^+$depleted cells							X	+/-[1]	+/-[2]	+/-[2]
Donor GCSF		X	X	X	X	X	+/-			
Donor apheresis						X	+/-	+/-[2]	+/-[2]	
Cell selection							X	+/-[1]		

1 An apheresis collection will be performed on day -1, and may also be performed on day 0. The selected product will be infused on day 0. (Also see section 9C, 9D &9E and Appendix A). 2On rare occasions an additional collection of PBSC, or bone marrow could be required.

Table 6. Treatment Plan Arm D (Lower-intensity conditioning, MUD)

Day of the week (*example one*)	**F**	**S**	**S**	**M**	**T**	**W**	**Th**	**F**	**S**	**S**
Day of the week (*example two)*	**T**	**F**	**S**	**S**	**M**	**T**	**W**	**Th**	**F**	**S**
Day relative to transplant of CD34$^+$ cells	**-6**	**-5**	**-4**	**-3**	**-2**	**-1**	**0**	**0/1**	**2**	**3**
Cyclophosphamide 50mg/kg x 1 day	X									

Thiotepa 5 mg/kg/day x 2 days		X	X							
Fludarabine 30 mg/m^2/day x5 days	X	X	X	X	X					
TBI 200cGy x 2 days					X	X				
Infusion of CD34$^+$ stem cells and CD45RA$^+$depleted cells							X	+/-[2]	+/-[2]	+/-[2]
Donor GCSF		X	X	X	X	X	+/-[1]	+/-		
Donor apheresis						X	+/-[2]	+/-[2]	+/-[2]	
Cell selection							X	+/-[2]		

[1] An apheresis will be collected on day -1, and transported as soon as possible on day-1. The selected product will be infused on day 0. [2] On infrequent occasions a second apheresis could be required to make the cell selection goals and would be collected on day+0, +1 or +2 and infused on day+1, +2 or +3. (Also see section 9C, 9D &9E and Appendix A).

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.

Intrathecal chemotherapy may be omitted if the attending physician considers that it is contraindicated for an individual patient based on prior toxicities or co-morbidities. No dose of intrathecal therapy will be given within 72 hours of CD34$^+$ stem cell infusion.

Male patients with ALL may be given a testicular radiation boost if it is considered indicated by the attending BMT physician and radiation oncologist. For patients with a history of CNS leukemia a CNS irradiation boost may also performed if it is clinically indicated in the opinion of the BMT physician and radiation oncologist.

9C. Collection of G-CSF mobilized PBSC (see also appendix A)

All donors will receive G-CSF administered by subcutaneous injection for 5 consecutive days on day -5 thru day -1, and possibly on D0. Related donors will receive 16 µg/kg/day and unrelated donors will receive 10 µg/kg/day. 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 should be confirmed with the personnel in the Cellular Therapy Laboratory. Typically, for URD transplant recipients the first apheresis would be performed on a Wednesday and transported to the SCCA Cellular Therapy Laboratory for cell selection on a Thursday.

PBSC collection by apheresis: Unrelated donors will undergo vein to vein apheresis collections. Related donors will typically undergo vein to vein apheresis unless their veins are considered inadequate and they are an adult in which case a central venous catheter may be inserted for apheresis collection. PBSCs will be collected using standard apheresis procedures on day -1. For related donors, and rarely for unrelated donors, a second collection may also be required on day 0 (see appendix A).

9D. PBSC graft engineering - CD34$^+$ cell selection and depletion of CD45RA$^+$ cells (see also appendix A)

- 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$^-$, CD3$^+$CD45RA$^-$ CD45RO$^+$ and total CD45RA$^+$ cells and an aliquot of cells will be obtained for

subsequent analysis of other cell subsets (see section 9D.3 below).

- We will perform cell selections *only* when the PBSC product from day -1, or day -1 and day 0 combined if applicable, contains ≥5x10^6 $CD34^+$ cells/kg of recipient body weight to ensure that the $CD34^+$ cell dose will be ≥2x10^6 $CD34^+$ cells/kg of recipient body weight. In most cases we expect that cell selections will be performed on a PBSC product from day -1 containing ≥8 x10^6 $CD34^+$ cells/kg of recipient body weight which should provide a $CD34^+$ cell dose of ≥5x10^6 $CD34^+$ cells/kg of recipient body weight.
- If the PBSC product(s) 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. We expect that ≤5% of patients enrolled on the protocol may receive a G-PBSC product that does not undergo any cell selection.
- We will perform cell selections only when the donor PBSC includes $CD3^+CD45RA^-$ $CD45RO^+$ cells. A small proportion of the normal population (3-4%) have a CD45 gene variant in which all the T cells express the high molecular weight isoform of CD45 and there is no distinct $CD45RO^+CD45RA^-$. CD45RA depletion will not be performed on such products as it would result in pan T cell depletion.
- For the patients in whom cell selection is performed we expect to achieve:
  - The CD34 goal of a minimum of 2.0 x 10^6 $CD34^+$ cells/kg of recipient body weight in all patients
  - A maximum of 5 x 10^4 $CD45RA^+RO^-CD3^+$ T cells/kg recipient body weight and a range of 1-10 x 10^6/kg total $CD3^+$ T cells in >95% of patients.
- Cell processing would commence ideally within 24 hours of receiving an apheresis, but may be delayed for up to 48 hours after collection if absolutely necessary.
- Timing (for additional details please see Appendix A):
  - The first apheresis would be performed on day -1, and transported as soon as possible on day -1.
  - A second apheresis may be performed for related donors, and rarely for unrelated donors on day 0.
  - Cell selections will be generally performed on day 0 using cells from the day -1 apheresis alone or in combination with cells from a day 0 apheresis collection.
  - In most cases the $CD34^+$ stem cell and CD45RA-depleted PBSC infusions would be administered on day 0.
  - On rare occasions $CD34^+$ cell target numbers may not be achieved and a second PBSC collection will be required and infused on day 1-3.

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, 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 Procedures. The goal is to obtain a target CD34 cell dose of >5.0x10^6/kg (minimum of >2.0x10^6/kg) of recipient body weight, 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 and typically results in a median $CD3^+$ cell dose of 2 x10^4/kg of which ≤ 30% are $CD45RA^+RO^-$.

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). We will plan to administer a total $CD3^+$ T cell dose of 10 $x10^6$/kg to the patient including both the $CD34^+$ enriched fraction and the CD45RA depleted fraction. 10 $x10^6$/kg total T cells is highly likely to contain < 5 $x10^4$/kg of $CD3^+CD45RA^+RO^-$ cells/kg on the basis of our experience to date. If necessary the total T cell dose may be reduced to maintain a maximum of 5 $x10^4$/kg of $CD3^+CD45RA^+RO^-$ cells/kg.

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 may be evaluated such as
         - $CD3^+$ $CD45RA^+$ $CD45RO^-$ $CCR7^+$ (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-7^+ (T_{CM}) and $CD3^+$ $CD45RO^+$ CCR-7^- (T_{EM})
         - $CD8^+$ $CD45RO^+$ CCR-7^+ (CD8 T_{CM}) and $CD8^+$ $CD45RO^+$ CCR-7^- (CD8 T_{EM}), and $CD4^+$ $CD45RO^+$ CCR-7^+ (CD4 T_{CM}) and $CD4^+$ $CD45RO^+$ CCR-7^- (CD4 T_{EM})
         - $CD3^{+/-}$ $CD56^+$, $CD3^{+/-}$ $CD16^+$, $CD14^+$, $CD19^+$ and $CD20^+$
         - $CD4^+$ $CD25^+FoxP3^+$.
   e. Specific T cell responses 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 product and CD45RA-depleted product will be infused through a central venous catheter on day 0 of the transplant (see Appendix D). The $CD34^+$ product will be infused first followed as soon as possible by the CD45RA-depleted product. In the very unlikely event that additional $CD34^+$ cells are required (see section 9D.1 and Appendix A and D), selected $CD34^+$ cells and/or $CD45RA^-$depleted cells and/or unselected PBSC will be administered through the central venous catheter on day 1-3.

9F. Post-transplant immunosuppression

GVHD prophylaxis will consist of tacrolimus and methotrexate for Arms A and C and tacrolimus and mycophenolate mofetil for Arms B and D. Tacrolimus (FK506) will be started on day -1 and should be continued for 50 days followed by a standard taper if there is no GVHD. For patients in Arm A and C methotrexate should be administered on day +1, +3, +6, +11. For patients in Arm B and D mycophenolate mofetil should be started on day -3 and continued until approximately day 30 and then discontinued with/without a taper at the discretion of the attending physician.

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. 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 20% of the day 50 dose per month for capsules (or 5% of the day 50 dose each week for liquid).
   c. If there is evidence of acute GVHD, then tapering of immunosuppression should be occur after resolution of symptoms according to the recommendations of the attending physician or institutional practice.
   d. If there is evidence of disease progression and no evidence of GVHD prior to day 50, patients 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. It is recommended that tacrolimus levels are maintained in the range of 5-15 ng/ml. It is recommended that whole blood trough levels are 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 recommended if the levels are outside the therapeutic range, or if there is evidence of toxicity that may be related to tacrolimus.
   b. 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.
   c. Blood pressure, renal function tests (creatinine, BUN), electrolytes and magnesium should be monitored regularly as per institutional standard practice guidelines.

3. Methotrexate administration (Arms A and C, High-intensity regimen)

   Methotrexate should be administered by IV push on days +1, +3, +6 and +11 at a dose of 5 mg/m^2. Calculation of m^2 should be according to institutional standard practice guidelines. Children weighing 10kg or less will have methotrexate dosed per kg rather than per /m^2 and should receive 0.165mg/kg per dose on days +1, +3, +6 and +11. The first dose of methotrexate should be given at approximately 24 hours, but no sooner than 24 hours after completion of the last cell infusion. If cells are given between days 1-3 (see

above), MTX is timed to the last cell infusion. The day 11 methotrexate dose may be omitted if the patient develops severe mucositis, a pleural effusion, ascites, or another relative contraindication to methotrexate administration.

4. Mycophenolate mofetil (MMF) administration (Arms B and D, lower-intensity regimen)
MMF should be administered starting on day -3 at the dose of 15 mg/kg every eight hours (based on adjusted body weight-see appendix I) with a maximum of 1 gram/dose. If actual body weight is <ideal weight dose according to actual body weight. The IV route should be used until day 10 and then, if tolerated the patient may change to PO beginning on day 11. Rounding of the dose to the nearest vial or capsule size is allowed. MMF will be given every eight hours until approximately day 30 post-transplant and then discontinued with/without a taper at the discretion of the attending physician. MMF should be continued or resumed beyond day 30 if donor chimerism is low (approximately <40% donor $CD3^+$ T cells) after discussion with the principal investigator.

9G. 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 or in the event of infectious complications as indicated in Section 12 E.

10. Evaluation

10A. Donor evaluation
Pre-donation evaluation for **unrelated donors** should be conducted as per NMDP (National Marrow Donor Program) standard procedure.

Related donors evaluation: The following should be obtained for all related donors:

1. 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.
2. CMV and EBV serologies performed within 30 days of donation.
3. Serum pregnancy qualitative within 2 weeks of the start of mobilization.
4. G-CSF and monitoring blood draws (including CBC and Hepatic Function Panel with LDH) should be conducted per institutional standard practice guidelines.
5. Research Tests: A 90 cc sample of related donor blood should be collected (preferably before the donor starts GCSF) in sodium heparin 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 and sent to the Bleakley lab.

10B. Patient pre-transplant evaluation
Patient pre-transplant evaluation should be conducted as per standard institutional practice. Results of tests and/or procedures conducted as part of that evaluation may be used for eligibility determination. It is recommended that the following information 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. ABO and Rh typing and two-way red cell cross match between recipient and donor as per standard practice.
   f. 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 quantitation within 30 days of the start of conditioning.
   g. CMV, EBV, HSV, VZV serologies within 30 days of the start of conditioning.
   h. CMV and EBV (and ADV for patients <18 years old) PCR obtained and run within 2 weeks prior to starting conditioning.
   i. CBC within 2 weeks of the start of conditioning.
   j. LFTs, to include ALT, AST, and Bili T/D, within 2 weeks of the start of conditioning.
   k. Serum chemistry, to include Na, K, Cl, CO_2, BUN, Cr, Ca, Mg, and Phos, within 2 weeks of the start of conditioning
   l. Serum pregnancy qualitative testing within 2 weeks of the start of conditioning in females of childbearing age.
3. Other evaluations:
   a. Echocardiogram or MUGA within one month of the start of conditioning.
   b. Pulmonary function tests, including measurement of DLCO (or documented 0_2 saturation on room air for patients < 6 years or considered developmentally incapable of PFTs by attending physician and PFT lab technician)
4. Research samples

   Peripheral blood. A 90 ml peripheral blood sample (1 ml/kg, up to 50 mL, for patients <18 years old) in sodium heparin or ACD should be sent to the Bleakley lab for subsequent use in evaluation of antigen-specific T cells responses after HCT.

10C. Patient post transplant evaluation guidelines

For FHCRC patients, see Standard Practice Manual for standard evaluation procedures during the first 100 days post transplant, evaluation prior to departure and long-term follow-up. Post transplant evaluation prior to day 80 will in most cases be performed whilst the patient is under the care of the BMT team. After day 80 evaluations may be performed under the supervision of the BMT team and/or long-term follow-up (LTFU) and/or the patient's local physician. Specific recommendations (which may be modified by the clinical team as deemed clinically appropriate) include:

1. It is recommended that bone marrow aspiration is performed at baseline and on approximately days +28, and between approximately day + 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. It is recommended that donor and recipient chimerism of $CD3^+$ and $CD33^+$ subsets in the peripheral blood 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. It is recommended that quantitative immunoglobulins are assessed at approximately days +28, +56 and + 80 and if possible on approximately day +180 and +360.
4. CMV and EBV (and ADV for patients <18 years old), monitoring is recommended for all patients. Specifically it is recommended that:
    a. CMV PCR should be performed at least once every week until day 100 according to institutional standard practice. Preemptive antiviral therapy should be instituted for a positive PCR according to institutional guidelines for recipients of T cell depleted allogeneic HCT (or an IRB approved research protocol for CMV management). CMV PCR monitoring is suggested to continue 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.
    b. Surveillance for EBV reactivation should be performed using a quantitative EBV DNA PCR assay approximately weekly until day 100. Infectious disease consultation should be obtained for patients who develop EBV DNA levels of >1000 copies/ml plasma on any test.
    c. For patients < 18 years old, surveillance for Adenovirus reactivation should be performed using a quantitative PCR assay (including adenovirus AF, and BCDE serogroups) approximately weekly until D180. Infectious disease consultation should be obtained for patients who develop Adenovirus DNA levels of >300 copies/mL plasma on any test.
5. Research Tests. **Please note that all research orders will be proposed by a member of the research team. Clinical providers should not order research samples that have not been proposed and should contact the research team if questions arise.**
    a. The following should be collected on approximately days +28, +56 and + 80 and if possible on approximately day +180 and +360.
        i. It is recommended that a peripheral blood sample, up to 4 mL, is obtained (and sent to SCCA/UWMC Hematopathology lab) for lymphocyte subset evaluation on approximately days +28, +56 and + 80 and if possible on approximately day +180 and +360. Specifically, it is recommended that the following subsets are enumerated: $CD3^+$, $CD3^+CD4^+$, $CD3^+CD8^+$, $CD4^+T_N$ ($CD3^+/CD4/CD45RA^+/CD45RO^-/CD62L^+$) and $CD8^+T_N$ ($CD3^+/CD8^+/CD45RA^+/CD45RO^-/CD62L^+$), NK ($CD3^-CD56^+CD16^+$), B cells ($CD19^+$), Tregs ($CD4^+/CD25^+/FOXP3^+/CD127^-$).
        ii. A 90 ml sample (1 ml/kg, up to 50 mL, for patients <18 years old)of peripheral blood should be collected in sodium heparin or ACD tubes and sent to the Bleakley lab for research tests which may include:
            1. TCR Vβ sequencing.
            2. T cell specificity evaluation including detection of minor histocompatibility antigen specific T cells.
            3. T cell functional immune reconstitution assays.
        iii. A sample up to 10 mL (2 mL for patients <18 years old) of peripheral blood may be sent in serum or plasma separating tubes to the Bleakley lab for cytokine and protein studies once pre-treatment and up to three times a week from start of conditioning until day +28, up to once per week from day 28 to approximately day 100 and then once at approximately day 180 and once at approximately day 360.

Peripheral blood research samples may be deferred or declined by the patient or attending physician depending on the patient's clinical status and preferences. The research tests performed will be prioritized by the PI depending on the number of cells available.

A summary of recommended patient evaluations is provided in Table 4.

Table 4. Patient evaluations over the course of the study

Approximate days in relationship to transplant

Study Assessments/ Testing	Baseline	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	X
Karnofsky/Lansky 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	X	X	X	X	X	X	X	X	X	X	X	X	Weekly to day 100		
EBV PCR	X	X	X	X	X	X	X	X	X	X	X	X	Weekly to day 100		
Quantitative Immunoglobulins for IgG, IgA and IgM					X				X				X	X	X
Peripheral blood T cell & myeloid chimerism ($CD3^+$ & $CD33^+$)	X Baseline DNA				X				X				X	X	X
Bone marrow aspirate/biopsy[3]	X				X								X		X
Acute GVHD		X	X	X	X	X	X	X	X	X	X	X	X		
Chronic GVHD[4]													X		X
Pulmonary function test including DLCO	X												X		X
Research Tests[5]															
Peripheral blood	X				X				X				X	X	X
Quantitative blood lymphocyte subset research evaluation					X				X				X	X	X

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). There may also be variation in the content of the evaluation performed particularly beyond day 100 depending on the patient's health status, size, geographical location, physician and/or PI recommendations and unanticipated variables. CLIA certificates will not be collected from outside labs for time-points beyond day 100.

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. Bone marrow aspiration; evaluation with **a.** histopathology **b**. flow cytometry **c.** cytogenetics and/or molecular testing where clinically relevant (FISH and PCR informative for disease status e.g. BCR-ABL for Ph+ ALL)
4. Chronic GVHD evaluation should be performed between approximately day 80 and 100 and on 360 where possible. Day 360 evaluations should be performed at the SCCA or UPMC if possible, or patients may have a 'home town evaluation' conducted by their regular medical provider with guidance by LTFU or UPMC transplant physicians as appropriate. 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 evaluated by the LTFU team at the SCCA or using telemedicine and/or medical photography in consultation with the patients regular medical provider where possible. Chronic GVHD evaluations at UPMC will be conducted by the UPMC transplant team.
5. Research samples may be deferred or declined by the patient, their guardian or attending physician depending on the patient's clinical status and preferences. The research tests will be prioritized by the PI depending on the number of available cells and the patient's clinical course.
6. **Please note that all research orders will be proposed by a member of the research team. Clinical providers should not order research samples that have not been proposed and should contact the research team if questions arise.**

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. Arm A and C: TBI will be given as 165 cGy fractions twice per day x 4 days (days -10 to -7) to patients on Arm A and C using the linear accelerator at a rate of 6-15 cGy/min.
2. Arm B and D: TBI will be given as 200 cGy fractions once per day x 2 days (days -2, -1) to patients on Arm B and D using the linear accelerator at a rate of 6-25 cGy/min.
3. Dosimetry calculations will be performed by the radiation oncologist.
4. 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, secondary malignancies, scoliosis, neurocognitive effects, renal dysfunction, and 'metabolic syndrome' (high blood pressure, abnormal blood lipid levels, high blood sugar levels and risk for diabetes).

11B. Cyclophosphamide Arms B and D

Cyclophosphamide 50 mg/kg/day (adjusted body weight-see appendix I) x 1 day IV (day -6) will be given to patients on Arms B and D. Cyclophosphamide will be administered intravenously over 1 hour. Mesna at a dose of 100% of the cyclophosphamide dose in four divided doses will be administered according to institutional standard practice. Patients will receive intravenous hydration for 8-10 hours (for example 2 hours preceding and 8 hours following cyclophosphamide) according to institutional standard practice. Toxicity:

a. Nausea, vomiting, diarrhea
b. Mucositis
c. Blood count suppression
d. Hemorrhagic cystitis
e. Sterility
f. Fluid weight gain or edema
g. Uncommonly cardiomyopathy, skin rash, syndrome of inappropriate anti-diuretic hormone suppression.

11C. 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 (Arms A and C day -6 and day -5, Arms B and D -5 and -4). 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.

11D. Fludarabine

1. Dosage

Arms A and C: 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.

Arms B and D: Fludarabine will be administered in a dose of **30** 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 150 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.

11E. PBSC infusions

Refer to Appendix D for infusion of selected cells.

11F. Tacrolimus administration

1. Also see section 9F for information about tacrolimus administration and dosage adjustments.
2. Administration and dosage
   a. Intravenous dosing - 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. A tacrolimus taper may be started on ≥ day 50 in the absence of active GVHD.
   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. Children < 6 years old may require oral dosing every eight hours to maintain target serum trough levels.

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
h. Diabetes

11G. Methotrexate administration

1. Also see section 9F for information about methotrexate administration and dosage adjustments.
2. Administration and dosage
   a. Methotrexate should be administered by IV push on days +1, +3, +6 and +11 at a dose of 5 mg/m^2. Calculation of m^2 should be according to institutional standard practice guidelines. Children weighing 10kg or less will have methotrexate dosed per kg rather than per /m^2 and should receive 0.165mg/kg per dose on days +1, +3, +6 and +11.
   b. The first dose of methotrexate should be given at approximately 24 hours, but no sooner than 24 hours after completion of the cell infusion.
   c. If clinically indicated methotrexate levels may be obtained and/or methotrexate dose adjusted according to institutional standard practice guidelines and/or leucovorin rescue may be considered. In the case of severe mucositis or in the presence of effusions, ascites, or another relative contraindication to methotrexate administration, the day +11 methotrexate may be withheld at the discretion of the attending physician and after communication with the principal investigator.
3. Toxicity
   a. In this setting of HCT, mucositis is the primary toxicity related to methotrexate.
   b. If there is also renal dysfunction methotrexate clearance may be delayed.
   c. Methotrexate may cause an elevation in serum transaminases.
   d. Severe skin reactions after single or multiple doses of methotrexate have occasionally been reported.

11H. Mycophenolate Mofetil Administration

MMF should be administered starting on day -3 at the dose of 15 mg/kg every eight hours (based on adjusted body weight-see appendix I) with a maximum of 1 gram/dose. If actual

body weight is <ideal weight dose according to actual body weight. The IV route should be used until day 10 and then, if tolerated the patient may change to PO beginning on day 11. Rounding of the dose to the nearest vial or capsule size is allowed. MMF will be given every eight hours until approximately day 30 post-transplant and then discontinued with/without taper at the discretion of the attending physician. MMF should be continued or resumed beyond day 30 if donor chimerism is low (<40% donor $CD3^+$ T cells) after discussion with the principal investigator. Toxicities:

a. Nausea, vomiting and diarrhea
b. Low blood counts
c. Infection
d. Headache
e. Hypertension
f. Dizziness
g. Insomnia
h. Electrolyte imbalances
i. Leg cramps, bone pain
j. Hyperglycemia
k. Rash
l. Spontaneous abortion
m. Birth defects.
n. Progressive multifocal leukoencephalopathy.

12. Protocol Enrollment and Special Considerations

The protocol will be open to accrual until 80 patients have enrolled and received a naïve T cell depleted product. In rare situations, patients may not receive a naïve T cell depleted product (such as low total CD34+ cell collection or an unexpected change in donor). For the purposes of total enrollment, patients that do not receive naïve T cell depleted products will not be included in the accrual numbers (80 total patients). All patients, however, that enroll and receive treatment on the protocol, will be monitored for safety (**Section 15**).

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	31	46	78
Ethnic Category Total of All Subjects*	33	47	80
Racial Categories			
American Indian / Alaska Native	1	1	2
Asian	1	1	2
Native Hawaiian or Other Pacific Islander	1	1	2
Black or African American	1	1	2

White	29	43	72
Racial Categories: Total of All Subjects*	33	47	80

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. 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) ***UPMC Standard:*** Ceftriaxone 1gm IV Q24 beginning day -2 until day 10.
      (iii) ***If Penicllin Allergic***: IV Vancomycin 15 mg/kg IV every 12 hours from day -2 through day +10
      (iv)***Alternative prophylactic regimens covering Streptococcus viridans are acceptable***.
   (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 or an alternative antifungal medication should be used from the first day of conditioning or sooner until day +75 post transplant in accordance with standard practice (or according to an IRB approved anti-fungal research protocol)
3) Preemptive therapy for CMV reactivation should be administered according to institutional standard practice guidelines for a T cell depleted allogeneic HCT (or according to an IRB approved CMV research protocol).
4) Infectious disease consultation should be obtained for patients who develop EBV DNA levels of >1000 copies/ml plasma on any test.
5) Infectious disease consultation should be obtained for patients who develop Adenoviral DNA levels of >300 copies/mL plasma on any test.(during weekly monitoring for patients <18 years old)
6) Patients should receive acyclovir or valacyclovir 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 and ≥20-30 days after HCT, patients may receive prophylaxis for pneumocystis carinii according to institutional standard practice guidelines.

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

1. UDCA is recommended to prevent hepatic complications according to standard practice guidelines.

12D. Management of acute and chronic GVHD

1. Patients who develop ≥ grade II acute GVHD should be treated with systemic

corticosteroids and/or 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, monoclonal antibodies, pentostatin, thalidomide and extracorporeal photopheresis, given on or off study protocols.

2. Patients who develop chronic GVHD and require systemic immunosuppressant therapy 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, small molecule inhibitors and/or donor lymphocyte infusions (DLI), or may be eligible for research protocols including 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, University of Pittsburgh Cancer Institute and the University of Pittsburgh Medical Center.

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 by the FHCRC principal investigator.

For University of Pittsburgh patients, primary research data may also be collected and maintained by the data coordinator and stored at the University of Pittsburgh Cancer Institute Clinical Research Services (CRS) in a limited access department. 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 in patients using PBSC that are selectively depleted of $CD45RA^+$ T cells.

14B. Definition of endpoints

1. Chronic GVHD
   a. Definition: Chronic GVHD will be diagnosed using NIH criteria outlined in Appendix F. Chronic GVHD will be defined operationally as the occurrence of compatible symptoms.
   b. Evaluation will be conducted BMT/LTFU team at day 80-100 and where possible on approximately day 360 and yearly up to 5 years after HCT. The patient's primary care physician and/or oncologist will conduct clinical assessments between these time points as required. 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 evaluated by the LTFU team at the SCCA or UPMC or using telemedicine and/or medical photography in consultation with the patients regular medical provider where possible.
   c. Endpoints
      i. The occurrence of chronic GHVD meeting NIH criteria and requiring systemic pharmacological immunosuppression (systemic corticosteroids and/or other systemic agents, excluding calcineurin inhibitors which are generally not considered adequate as sole agents for the treatment of chronic GVHD)
      ii. The use of additional immune suppressive agents other than first line therapy (first line therapy is considered prednisone and tacrolimus/cyclosporin).
      iii. Time to completion of prednisone
      iv. Time to completion of all immunosuppression
      v. Requirement for immunosuppression at 2 years after transplant.
2. 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. Requirement for secondary systemic therapy for acute GVHD management

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 necessarily 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 platelet count >20,000/µL for 3 days without transfusion.
      iv. Time to platelet count >50,000/µL for 3 days without transfusion.
      v. It is recommended that chimerism analysis of CD3 and CD33 cells in peripheral blood be performed on or around day 28, 56, 80-100, 180, and 360. Additional peripheral blood or marrow chimerism studies may be performed as clinically indicated.
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 4. Suspected extramedullary sites of recurrent disease may be evaluated by biopsy and/or lumbar puncture if clinically indicated.
4. Transplant related mortality (TRM). TRM is defined as mortality in any patient for whom there has not been a diagnosis of relapse.

14C. <u>Statistical Analysis and Stopping Rules</u>

a. Chronic GVHD

The first primary objective is to estimate the probability of developing chronic GVHD (NIH criteria). MRD-high intensity (Arm A), MRD-lower intensity (Arm B), MUD-high intensity (Arm C) and MUD-lower intensity (Arm D) cohorts will be analyzed separately. Published data from FHCRC examining the outcome of patients who received myeloablative MUD (10/10 allele) or MRD HCT and GVHD prophylaxis with a calcineurin inhibitor and methotrexate between 1992 and 2008 shows a rate of 45% chronic GVHD among 563 patients with MUD donors and 42% among 885 patients with MRD [4]. In contrast, amongst the first cohort of patients treated on a clinical trial of MRD T_N-depleted PBSCT (FHCRC protocol 2222), with a median follow-up of >590 days the cumulative incidence of chronic GVHD was 12% at 2 years (95% confidence interval 0-26%). The use of high-dose TBI-containing conditioning may be a risk factor for the development of chronic GVHD [46,47]. We will therefore determine whether:

a. Patients who receive T_N-depleted HCT from a MRD following high-intensity myeloablative conditioning followed by tac/MTX (Arm A) have a lower frequency of chronic GVHD as compared to historical controls. If the true rate of chronic GVHD is 17% in recipients of T_N-depleted tac/MTX MRD HCT then 20 patients will provide 90% power to observe a statistically significantly reduced rate (at 1-sided level of 0.10) compared to the fixed benchmark of 42%.

b. Patients who receive T_N-depleted HCT from a MRD following lower-intensity myeloablative conditioning followed by tac/MMF (Arm B) have a lower frequency of chronic GVHD as compared to historical controls. If the true rate of chronic GVHD is 17% in recipients of T_N-depleted tac/MMF MRD HCT then 20 patients will provide 90% power to observe a statistically significantly reduced rate (at 1-sided level of 0.10) compared to the fixed benchmark of 42%.

c. Patients who receive T_N-depleted HCT from a MUD following high-intensity myeloablative conditioning followed by tac/MTX (Arm C) have a lower frequency of chronic GVHD as compared to historical controls. If the true rate of chronic GVHD is 19% in recipients of T_N-depleted tac/MTX MUD HCT then 20 patients will provide 90% power to observe a statistically significantly reduced rate (at 1-sided level of 0.10) compared to the fixed benchmark of 45%.

d. Patients who receive T_N-depleted HCT from a MUD following lower-intensity myeloablative conditioning followed by tac/MMF (Arm D) have a lower frequency of chronic GVHD as compared to historical controls. If the true rate of chronic GVHD is 19% in recipients of T_N-depleted tac/MMF MUD HCT then 20 patients will provide 90% power to observe a statistically significantly reduced rate (at 1-sided level of 0.10) compared to the fixed benchmark of 45%.

2. Acute GVHD

A second primary objective of this single-arm Phase II study is to estimate the probability of grades II-IV acute GVHD. The goal is to observe a statistically significant reduction in the probability of GVHD with T_N-depleted PBSCT with high-intensity conditioning and tac/MTX in Arm A and with lower intensity conditioning and tac/MMF in Arm B, each compared to the historical experience with T_N-depleted PBSCT with tacrolimus alone. The frequency of aGVHD grade II-IV among recipients of MRD T_N-depleted PBSCT followed by tacrolimus monotherapy is (67%, 95% confidence interval (CI) 50-84%). We will determine whether:

a. Patients who receive MRD T_N-depleted PBSCT from a MRD following high-intensity myeloablative conditioning (Arm A) followed by tac/MTX have a lower frequency of GVHD as compared to these historical controls. If the true rate of aGVHD is 39% in recipients of T_N-depleted HCT in Arm A then 20 patients will provide 90% power to observe a statistically significantly reduced rate (at 1-sided level of 0.10) compared to the fixed benchmark of 67%.

b. Patients who receive MRD T_N-depleted PBSCT from a MRD following lower-intensity myeloablative conditioning (Arm B) followed by tac/MMF have a lower frequency of GVHD as compared to these historical controls. If the true rate of aGVHD is 39% in recipients of T_N-depleted HCT in Arm B then 20 patients will provide 90% power to observe a statistically significantly reduced rate (at 1-sided level of 0.10) compared to the fixed benchmark of 67%.

MUD recipients were not included in our first clinical trial of T_N-depleted HCT and we therefore do not have historical benchmark data for GVHD rates in MUD T_N PBSCT with tacrolimus monotherapy. However, given that the rates of aGVHD are

generally consistently similar or slightly higher in MUD HCT compared to MRD HCT, we will use the same benchmark for comparison.

c. If the true rate of aGVHD is 39% in recipients of T_N-depleted HCT in Arm C (MUD donor, high intensity conditioning, tac/MTX) then 20 patients will provide 90% power to observe a statistically significantly reduced rate (at 1-sided level of 0.10) compared to the fixed benchmark of 67%.
d. If the true rate of aGVHD is 39% in recipients of T_N-depleted HCT in Arm D (MUD donor, lower intensity conditioning, tac/MMF) then 20 patients will provide 90% power to observe a statistically significantly reduced rate (at 1-sided level of 0.10) compared to the fixed benchmark of 67%.

3. Graft failure will be closely monitored throughout this study. The MRD high-intensity, MRD lower-intensity, MUD higher intensity and MRD lower intensity cohorts will be treated separately. For each cohort a true probability of graft failure over 10% 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 8 patients, 3 among the first 15 patients, or 4 among the first 20 patients in any cohort, enrollment of patients in that cohort 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 10%. Demanding higher confidence that the estimated graft failure rate exceeds 10% before stopping means that the chances the trial would stop when the true graft failure rate is over 10% are unacceptably low. If the true failure rate is as low as 5%, then the probability that of the trial stopping after 20 patients is approximately .08. If the true failure rate is 25%, the probability of stopping is approximately .85.

4. Failure to deliver therapy
If the $CD34^+$ stem cells cannot be delivered as planned for the first 2 patients enrolled either the MRD cohorts (A+ B) or the MUD cohorts (C + D), or for any other two consecutive recipients of MRD or MUD PBSC, 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.

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.

When a patient withdraws consent from the protocol 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 unless they have signed the general FHCRC consent and authorization form allowing their leftover specimens and medical records to be used for research. If they have 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.

According to ICH guidelines and 21 CFR 312.32, IND Safety Reports, and ICH E2A, Definitions and Standards for Expedited Reporting, an adverse event is defined as follows:

An adverse event is any untoward medical occurrence in a clinical investigation subject administered a medicinal product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

Abnormal laboratory values for laboratory parameters specified in the study should not be recorded as an adverse event unless an intervention is required (repeat testing to confirm the abnormality is not considered intervention), the laboratory abnormality results in a serious adverse event or the adverse event results in study termination or interruption/discontinuation of study treatment.

Medical conditions present at screening (i.e., before the study treatment is administered) are not adverse events and should not be recorded on adverse event pages of the CRFs. These medical conditions should be adequately documented on the subject chart. However, medical conditions present at baseline that worsen in intensity or frequency during the treatment or post-treatment periods should be reported and recorded as adverse events.

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, with the exception of some abnormal lab values and medical conditions present at screening (as noted in section 15B)
2. Grade 3-5 Blood/Bone Marrow adverse events occurring between day 60 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.
5. All treatment related mortality attributed to the investigational cell product occurring in the first 100 days will be reported to the IRB and the FDA as stated in sections 15.E and

H.

6. Grade 3-5 infusion reactions attributed to the investigational cell product will be reported to the IRB and FDA within the time frame specified in sections 15.E and H.

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 UPMC patients will be reported by the UPMC investigator or representative to the FHCRC PI and/or study nurse. The trial coordinators at collaborating centers or the local PIs will report all events requiring expedited reporting (including, but not limited to, all patient deaths regardless of cause, occurring days 0-200 post-transplant procedure) by email – addressed to both the FHCRC PI and study nurse, within 72 hours of learning of the event. 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 (the FHCRC PI).

15E. Reporting Requirement to FHCRC IRB

The Principal Investigator, study nurse, or coordinator shall submit to the FHCRC IRB reportable events according to current reporting policies as outlined in FHCRC IRB Policies for Reportable Events.

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

fdsm

All reportable events should be submitted on the relevant FHCRC Forms (URLs linking to the FHCRC IRO website are found in Table 5 FHCRC IRB Forms for Reporting).

Table 5. 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 Exemptions (IDE)	http://extranet.fhcrc.org/EN/sections/iro/irb/policy/index.html

Table 6. 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 IND Sponsor

Grade 3-5 infusion reactions will be reported and sent via email to the sponsor, Dr. Marie (mbleakle@fhcrc.org) and the regulatory 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. Bleakley and the regulatory coordinator should be contacted again by email and additionally by telephone or FAX.

All serious adverse events (SAEs) should be reported via email to the sponsor. A single email notification should be sent to the Sponsor within 48 hours of the study team's awareness of any Serious Adverse Events. All events reported to the Sponsor by email that meet Serious, Unexpected and Related criteria must clearly specify that the adverse event meets expedited reporting requirements.

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, Dr. Marie Bleakley, as soon as possible but in no event later than 10 working days after the investigator first learns of the event. If a previously reported grade 3 SAE increases to grade 4 and does not resolve within 96 hours to grade 2, a second email should be sent to the sponsor.

15G. Reporting Requirement to University of Pittsburgh IRB

All reportable events whether originating at University of Pittsburgh or a collaborating center will be reported by University of Pittsburgh study staff to the University of Pittsburgh IRB per its reporting guidelines.

15H. Reporting to the FDA

As a study conducted under IND (Investigational New Drug) regulations we will comply with the FDA regulations regarding safety reporting 21CFR312.32 including the following requirements:

1. A sponsor must promptly review all information relevant to the safety of the drug 21CFR312.32 (b).
2. A sponsor must notify FDA in an IND safety report of potential serious risks, as soon as possible but in no case later than 15 calendar days after the sponsor determines that the information qualifies for reporting under 21 CFR312.32 (c)(1). Information that is required to be reported includes, but is not limited to, a. Serious and unexpected adverse reactions and b. An increased rate of occurrence of serious suspected adverse reactions.
3. The IND safety report must be completed and sent to the FDA in a narrative format, on FDA Form 3500A, or an electronic format.
4. A sponsor must also notify FDA of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible but in no case later than 7 calendar days after the sponsor's initial receipt of the information 21CFR312.32 (c)(2).

15I. Reporting to NIH National Heart, Lung and Blood Institute

As a study supported by the National Heart, Lung and Blood Institute we will comply with NHLBI reporting policies. Specifically, we will report the following events to NHLBI within the reporting policy timeframe (http://www.nhlbi.nih.gov/funding/policies/adverse.htm) as well as to the IRB and FDA as outlined in sections 15A to 15H:

1. The Investigator will notify NHLBI of any fatal or life-threatening unexpected, suspected related or probably related serious adverse reaction within 7 calendar days of initial receipt of the information.
2. The Investigator will notify NHLBI of any non-fatal, non-life-threatening unexpected, suspected related or probably related serious adverse reactions within 15 calendar days of initial receipt of the information.
3. Any unanticipated problem (defined as any incident, experience or outcome that is 1) unexpected 2) related or possibly related to participation in the research and 3) suggests that the research places subjects or others at greater risk of harm than was previously recognized - http://www.nhlbi.nih.gov/crg/glossary.php#unanticipated) that is not an SAE will be reported to NHLBI within 14 days of the investigator becoming aware of the problem.

16. Data safety monitoring plan

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

The FHCRC PI/Sponsor is responsible for monitoring this clinical trial, with oversight by a Data and Safety and Monitoring Board (DSMB), the Data Safety Monitoring Committee (DSMC) and the IRB at the FHCRC. 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 and 12 months after HCT, at a minimum.

A DSMB will be in place to meet approximately every 6 months to review the data particularly as it relates to engraftment, grades III-IV GVHD and relapse. To date five patients or less have been treated every six months. 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 FHCRC IRB and the PI. The DSMB will discontinue the review of outcomes when all subjects on this trial have completed all protocol-specified follow-up.

All members will have experience in the management of patients with leukemia and myelodysplasia and in the conduct and monitoring of clinical trials. At least some of the members of the DSMB will have experience in the management of pediatric patients with leukemia and myelodysplasia.

The DSMC at FHCRC 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.

A protocol monitor (from the FHCRC Clinical Research Support, or an external monitor) will be retained to monitor study progress. The scope of monitoring will be based on the FHCRC/UW Data and Safety Monitoring Plan:

http://centernet.fhcrc.org/CN/depts/iro/irb/dsm/. 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, and the Principal Investigator/Sponsor at FHCRC.

Flow of information concerning clinical trial participants originates with the clinicians and nurses in the clinic and is transmitted to the FHCRC Research Nurse. At the FHCRC and UPMC 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 that follows patients to confirm eligibility, reporting of adverse events, reporting of events which are part of the safety-monitoring plan, and protocol adherence. The PI 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 +130) and again at approximately 1 year (no later than 15 months) after HCT via chart review and entered into electronic protocol specific Case Report Forms (CRFs). The principal investigator will review the CRFs and the primary source documents verifying by signature (electronic) their data accuracy.

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.

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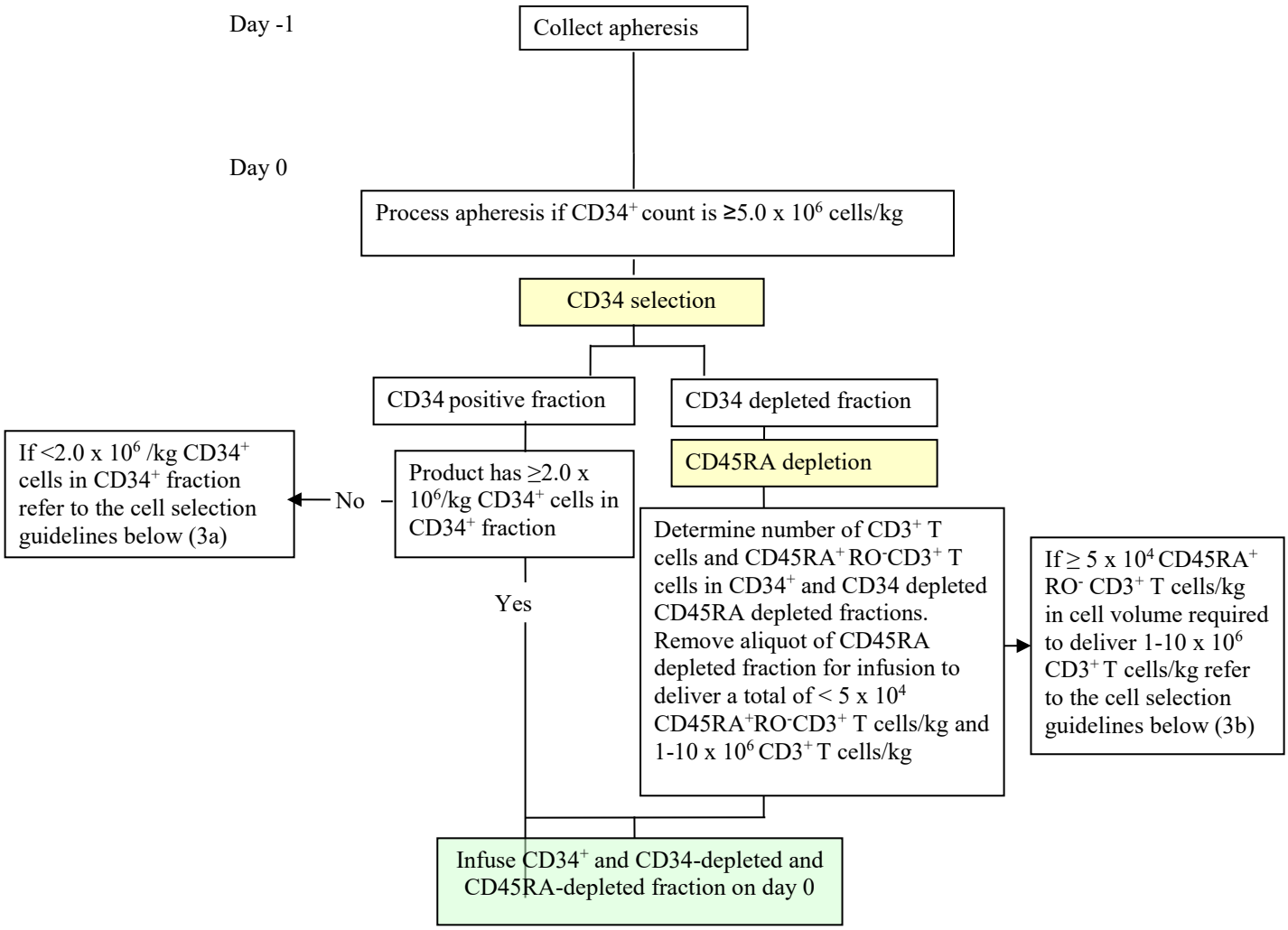
APPENDIX A
Cell Selection Flow Sheet



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

- A starting total CD34 cell dose of ≥8 x10^6 cells/kg will be available in most donors and a total $CD34^+$ cell dose of >2.0x10^6 cells/kg of recipient body weight (and in most cases >5.0x10^6 cells/kg) will be obtained following CD34 positive selection from an apheresis product containing 8 x10^6 $CD34^+$cells/kg. We expect to infuse 2-8 million $CD34^+$ cells/kg in most cases, and plan to limit the infused $CD34^+$ cell dose to no more than approximately 10 million $CD34^+$ cells/kg in most cases.
- 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 <1 x10^4/kg of recipient body weight.
- Depletion of $CD45RA^+$ cells from the CD34 negative fraction will yield sufficient T cells to administer 10 x10^6 $CD3^+$ cells/kg and <1x10^4 $CD45RA^+$ RO^- $CD3^+$ cells/kg of recipient body weight for >95% 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 5 x 10^4 $CD45RA^+RO^-CD3^+$ T cells/kg and the number infused is very likely to be <2x10^4 $CD45RA^+$ RO^- $CD3^+$ cells/kg.

<u>Cell Selection Guidelines (also see apheresis collection guidelines, below)</u>

1. We will perform cell selections only when the PBSC product from days -1 or day -1 and 0 combined contains ≥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.

2. We will also perform cell selection only when the T cells in the PBSC product include a distinct $CD45RO^+$, $CD45RA^-$ population observed by flow cytometry. ≤5% of donor products are expected to have a CD45 variant in which all the T cells express CD45RA and there is therefore no $CD45RO^+$, $CD45RA^-$ population. If there is no $CD45RO^+$, $CD45RA^-$ population the PBSCs will be infused without any cell selection and the patients will not be included in the analysis of study *efficacy* end points.

3. Situations may arise where the goals for cell selection are not achieved with cell processing. These very 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 ≥2.0 x 10^6 cells/kg is *not* achieved after the processing the apheresis collection(s) all available $CD34^+$ cells will be administered to the patient as soon as possible and an additional apheresis collection will be requested 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.

b) *If* < 5 x $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 x 10^6/kg $CD34^+$ cells and a range of 1-10 x 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 1 x 10^6 $CD3^+$ cells/kg and <5 x $10^4$$CD3^+$$CD45RA^+$ RO^- cell/kg cannot be achieved by i. then the patients should receive the CD34 selected product (minimum of 2.0 x 10^6 $CD34^+$ cells/kg) and may receive an aliquot of the $CD3^+$ cells (i.e. less than 1 x 10^6 $CD3^+$ cells/kg) containing <5 x $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 as described in the protocol unless they receive only the $CD34^+$ selected product and < 1 x 10^5 $CD3^+$ cells/kg in which case they will receive no immunosuppression.

4. In a typical case we expect to a. perform cell selections on the day -1 apheresis collection (unrelated donors, some related donors), or a product pooled from the day -1 and day 0 collection (some related donors), b. to use a portion of the apheresis collection(s) containing at least 8-10 x 10^6 $CD34^+$ cells/kg for the cell selections, c. to reserve at least 3-5 x 10^6 $CD34^+$ cells /kg as a back-up for infusion of unselected PBSC into the patient if unanticipated problems arise in the cell selection procedure, and d. (for URD recipients) to cryopreserve at least 1 x 10^7 $CD3^+$ cells/kg for potential future donor lymphocyte infusion (DLI). We also plan to cryopreserve any available additional unmanipulated PBSC left over from the cell processing requirements as a back-up in the unlikely event of graft failure. In the unlikely event that there are insufficient unmanipulated PBSC to cryopreserve as DLI for URD recipients we may instead cryopreserve aliquots of the CD34-depleted fraction to be used as DLI.

<u>Apheresis Collection Guidelines</u>

Collection of unrelated donor GCSF mobilized apheresis products will be managed by the National Marrow Donor Program. Collection of related donor GCSF mobilized apheresis products will be managed by the SCCA or UPMC apheresis center. The following represents a general guideline

<u>Unrelated donors</u>

1. A large volume apheresis collection (processing approximately 24L of blood) will be performed on day -1 and transported as soon as possible on day -1.

<u>Related donors</u>

1. A large volume apheresis collection (processing approximately 18L of blood) will be performed on day -1. The CD34 content will be evaluated on day-1.

a) If ≥ 15 million CD34 cells/kg have been obtained from the day -1 no further apheresis is required. Cell processing can commence on day 0 using 10 million CD34 cells/kg with 5 million CD34 cells/kg held in the event of cell processing problems, and cryopreserved if not required.

b) If <15 million CD34 cells/kg have been obtained from the day -1 collection a second collection will be obtained on day 0 to a maximum of 18L.

i. The second collection may be totaled with the day -1 apheresis for a pooled or two separate cell selection(s) on day 0 and/or day +1 (e.g. if the day -1 apheresis was ≤ 8 x 10^6 CD34/kg and the total of the day-1 and 0 aphereses is ≥ 5 x 10^6/kg)

ii. The second collection may also be held as a back-up in case of unanticipated problems in the cell selection procedure of the first apheresis and subsequently cryopreserved if not required for cell selection.

On very rare occasions the minimum $CD34^+$ cell target number of 2 x 10^6/kg may not be achieved before or after cell processing and an additional donor apheresis collection may be requested and infused without cell selections.

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 ($CD34^+$ and $CD45RA^-$) 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 the 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. FHCRC and UPMC have 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 from the $CD34^+$ fraction 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 x 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 before cryopreservation 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 5 x 10^4 $CD3^+CD45RA^+$ RO^-/kg and a range of 1-10 x 10^6 $CD3^+$ cells/kg in the sum of the CD45RA-depleted and $CD34^+$ cell products and a minimum of 2.0 x 10^6 $CD34^+$ cells/kg.

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 leukapheresis collection will be transported and stored for a total of up to 48 hours from apheresis collection at 4C prior to CD34 selection and CD45RA depletion. 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 of the $CD34^+$ cells and CD45RA-depleted cells will take place within 6 hours after completion and release of the cell products.

APPENDIX C
Positive Culture from Donor Product Action Plan

In the event that a sterility-testing culture sample turns positive (after the infusion has taken place), 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. For events occurring at FHCRC, the FHCRC PI will notify the FHCRC IRB, as soon as possible, but no later than 10 calendar days of finding out of the event and will notify the FDA as outlined in section 15.
4. After notification of the attending physician, the following actions will take place. For events occurring at the University of Pittsburgh/UPMC, the PI will notify the University of Pittsburgh IRB and the FHCRC PI/Sponsor. The FHCRC PI/Sponsor will notify the FHCRC IRB within 10 days, and the FDA as described in section 15.
    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 UPMC 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 UPMC, diphenhydramine may be given as a premedication as per UPMC standard practice.

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

Product Infusion Rate
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 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 UPMC 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 UPMC patients, the UPMC 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-NIH criteria

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 (congestive heart failure, pericardial effusion, pericarditis, EKG changes) is uncommonly associated with chemotherapy agents and TBI and these sequelae may prove lethal.

4. Pulmonary toxicity. Diffuse interstitial pneumonitis and diffuse alveolar hemorrhage of unknown etiology occurs with some regularity after BMT. Interstitial fibrosis occurs 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 and MTX 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 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. Late effects of total body irradiation include cataracts, growth failure, gonadal failure and sterility, hypothyroidism, pulmonary dysfunction, secondary malignancies, scoliosis, neurocognitive effects, renal dysfunction and ‘metabolic syndrome’ (high blood pressure, abnormal blood lipid levels, high blood sugar levels and risk for diabetes).

11. 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, microangiopathy and hemolytic uremic syndrome, 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/Lansky Performance Status Scale

KARNOFSKY PERFORMANCE STATUS SCALE (RECIPIENT ≥16 YEARS)

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

LANSKY PERFORMANCE STATUS SCALE (RECIPIENT <16 YEARS)

Percentage	
100	Fully active
90	Minor restriction in physically strenuous play
80	Restricted in strenuous play, tires more easily, otherwise active
70	Both restrictions of and less time spent in active play
60	Ambulatory up to 50% of the time, limited active play with assistance/supervision
50	Considerable assistance required for any active play, fully able to engage in quiet play
40	Able to initiate quiet play
30	Needs considerable assistance for quiet activity
20	Limited to very passive activity initiated by others (e.g. TV)
10	Completely disabled, not even passive play
0	Dead

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:

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

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 2684)

1.1 Total Body Irradiation –High-intensity Myeloablative (Arms A and C)

1.1.1 Patients
Patients 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 treated with high-intensity myeloablative condition 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 ≥ 6 MV 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 18 MV 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; each lung will be blocked with two half value layer lung blocks for four of the eight fractions and it is recommended that lung shielding be used for the first 4 fractions in patients treated in the standing position. 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.

2.0 Total Body Irradiation –Lower-intensity Myeloablative (Arms B and D)

2.1 Preparation:

All metal and tightly fitting garments, including undergarments, must be removed prior to irradiation. All ointments, creams, powders, deodorants, perfumes or lip balm (including all Radia-Care products) should also be removed.

2.2 Sequencing of TBI

Total body irradiation will be administered on day -2 and day -1 of the preparative regimen

2.3 Treatment Volume

The total body will be treated including the head and feet in one field when possible. 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).

2.4 TBI Dose Prescription

Treatment will be delivered by photons of appropriate MV energy. The dose will be 200cGy daily for two days (total 400cGy). Dose rate will be prescribed to be within the range of 6-25 cGy/min (with a hard stop at 25 cGy/min).

2.5 Shielding Technique

The radiation oncologist may use lung shielding with one half value layer lung blocks for each fraction if s/he and the transplant physician agree the patient is at increased risk of pulmonary morbidity.

Portal images confirming the accuracy of lung block placement will be performed prior to each fraction when lung blocks are employed.

Lung block placements will conform to the following guidelines. The lateral edges will be 1 – 1.5 cm from the inner border of the ribs, the inferior edges will be 1 – 1.5 cm from the dome of the apex of the diaphragm, 1 – 1.5 cm below the clavicles and the medial border 2 – 2.5 cm from the lateral edges of the thoracic vertebral bodies. If there is residual abnormal hilar adenopathy, then lung shields will be contoured to provide radiation coverage of the adenopathy with a margin of 1 – 1.5 cm about the hilar mass.

2.6 TBI Techniques

2.6.1 Each treatment fraction shall comprise at least 2 opposed beam pairs.

2.6.2 Bolus and/or compensator devices will be utilized to ensure uniform radiation dose delivery within 10% of the prescribed dose. Treatment technique selected by the radiation oncology attending will be based on patient's size and their ability to remain still in the treatment position.

2.6.3 Technique 1-AP/PA in the upright position

The patient will be treated AP/PA in an upright position in the standard UWMC TBI positioning device at extended distance. Treatment will be delivered with equally weighted parallel opposed fields, with each treatment including both AP and PA fields.

2.6.4 Technique 2- AP/PA fields in the reclining lateral decubitus position

Patients unable to be treated in the upright position, will be treated at the discretion of the radiation oncologist/physicist with equally weighted AP/PA parallel opposed fields at an extended distance in the lateral decubitus position.
For the decubitus position the patient will rest their head on their decubitus arm. Their other arm will be positioned at their side. The patient's back will be supported.

The patient will be positioned in the right lateral decubitus position for the AP beam and PA beams. The patient will then be positioned in the left lateral decubitus position for the AP beam and PA beams for the following fraction. The decubitus position will continue to alternate for each fraction.

2.6.5 Technique 3-Supine position

Patients unable to tolerate the lateral decubitus position can be treated with right/left lateral parallel opposed fields at an extended distance in the supine position with arms at their side on a treatment couch or gurney.

2.6.6 Simulations

Treatment technique 1 (AP/PA in the upright position), 2 (AP/PA fields in the reclining lateral decubitus position), and 3 (lateral fields in the supine position): simulation measurement of patient thicknesses at various anatomical levels will be done for both seated upright, lateral decubitus, and supine techniques prior to beginning treatment. These treatment options must be available in case a change in the patient's treatment technique is required mid-course of their TBI

Patient thickness is measured at least at the following anatomical levels
the head, neck, upper mediastinum, lower mediastinum, umbilicus, knee, and ankle.

2.0 Treatment dose calculations

2.1 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 (MU) 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 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, such as accounting for

actual patient thickness.

2.2 Prescription and dose monitoring points

The prescription point is defined at the umbilicus.

There are three dose monitoring points at the following levels- head, umbilicus, and ankle. (See 4.0 Dose Monitoring Points.)

The prescription point must receive **at least** the prescribed dose. MU correction is required if the expected dose measured by the diodes at the level of the prescription point is over 5% high. All other dose monitoring points must be within +/- 10% of the dose prescription. An

AP and PA measurement of dose is required at the level of the prescription point so that MU changes, if required, may be made following the completion of the first treatment beam of the fraction.

No tissue inhomogeneity corrections will be made in the calculation of the dose to the prescription point.

Measurements and calculations of the required monitor units necessary for each treatment will be performed for the selected treatment technique (see section 1.5).

3.0 Dose compensation

Dose compensation is designed to achieve the prescription dose for the 3 dose monitoring points referenced below (4.1 through 4.3). The midline dose at these locations is recorded. The dose compensation is calculated based on the patient thickness at each anatomical level and factors appropriate to the TBI treatment conditions. It is recommended that entrance and exit dose detectors be placed on the patient at each anatomical level required as a dose monitoring point and the midline dose computed from these measurements.

4.0 Dose monitoring points

There are 3 dose monitoring points at the following levels. (Diodes are preferred.)

4.1 Head/Cranial ("Point 1"): This prescription point /dose monitoring 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. Prescription point measured each fraction AP/PA.

4.2 Umbilicus ("Point 2"): The prescription point/dose monitoring 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 beam. Prescription point measured each fraction AP/PA.

4.3 Ankle ("Point 3"): This dose monitoring point is defined along the midline at the midplane of the ankle at the level of the lateral malleolus.

5.0 Dose Uniformity Criteria

5.1 The objective is to keep the bone marrow and midline dose throughout the body to be at least 90% of the prescription dose

5.2 The dose uniformity as measured by the dose to the above reference points (#1,2,3) shall be kept within +/- 10% of the prescription dose.

5.3 If necessary, the treatment technique should be modified using tissue compensators in order to achieve the required uniformity.

5.4 Beam spoilers or other equally effective beam modifying devices are used to raise the superficial/skin dose to be at least 90% of the to the prescription dose.

5.5 The radiation detectors will be used at the monitoring points for at least one fraction. Compensators and monitor unit settings for radiation dose will be adjusted to ensure that the patient receives the prescribed dose within +/- 10% at all monitoring points # 1-3.

6.0 Additional Testicular Radiation

Testicular boost radiation may be delivered to selected male patients.

The dose of irradiation to the testicular area will be a total dose of 1600 cGy in 200 cGy fractions and be delivered by megavoltage electrons with the patient supine on the treatment couch, to a direct enface field encompassing the entire scrotal contents. The dose will be calculated to the 90% isodose at the depth of the posterior extent of the testes.

7.0 Additional Cranial or Craniospinal Radiation

In some cases of CNS involvement, additional cranial or craniospinal radiation may be required. The decision to give additional radiation will be determined by the radiation oncologist after discussion with the transplant attending.

8.0 Questions

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

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9.0 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 differs from the protocol dose by more than 10% but less than 20%.

Major Deviation:

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