A Single Arm, Multicenter Phase II Trial of Transplants of HLA-Matched, CD34$^+$ Enriched, T cell Depleted Peripheral Blood Stem Cells Isolated by the CliniMACS System in the Treatment of Patients with AML in First or Second Morphologic Complete Remission

BMT CTN PROTOCOL 0303
VERSION 7.0

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PROTOCOL SYNOPSIS – BMT CTN PROTOCOL 0303

A Single Arm, Multicenter Phase II Trial of Transplants of HLA-Matched, $CD34^+$ Enriched, T cell Depleted Peripheral Blood Stem Cells Isolated by the CliniMACS System in the Treatment of Patients with AML in First or Second Morphologic Complete Remission

Co-Principal Investigators: Richard O'Reilly, M.D., Steven Devine, M.D.

Primary Objective: The primary objective is to assess disease-free survival (DFS) at 6 months post-transplant. Death or relapse will be considered events for this endpoint.

Secondary Objectives: Secondary objectives are to assess infusional toxicity, the time to neutrophil and platelet engraftment, the incidence and severity of acute and chronic GVHD, the incidence of transplant-related mortality, the incidence of EBV post-transplant lymphoproliferative disorder, the time to leukemia relapse, the probability of survival and disease-free survival at 2 years post-transplant and the proportion of grafts with both $> 5 \times 10^6$ /kg CD34 cells and $< 1 \times 10^5$ /kg CD3 cells.

Study Design: The study is a single arm Phase II, multicenter trial. It is designed to determine whether the anticipated endpoints for a T cell depleted transplant arm of a planned prospective randomized trial comparing T cell depleted and unmodified hematopoietic allografts are likely to be achieved in a multicenter study conducted by the Blood and Marrow Transplant Clinical Trials Network (BMT CTN or Network). The study population is patients with acute myeloid leukemia (AML) in first or second morphologic complete remission. The enrollment is 45 patients.

Based on published results of unmodified transplants from HLA-matched siblings applied to patients with AML in first or second morphologic complete remission, a significant improvement in results with a graft modified as specified in this protocol would be expected if DFS at 6 months was > 75%, the true incidence of transplant-related mortality at 1 year was < 30% and the DFS rate at 2 years was $\geq$ 70% for patients transplanted in first remission and > 60% for patients transplanted in second remission. Additional secondary endpoints include: graft failure rate, incidences of acute grade II-IV and chronic graft-versus-host disease (GVHD). Additionally, the trial will have target specific doses of $CD34^+$ progenitors and $CD3^+$ T cells to be obtained following fractionation with the CliniMACS system. Based on the results of this trial, a Phase III trial comparing T cell depleted peripheral blood stem cell transplants (PBSCT) with unmanipulated bone marrow or unmanipulated PBSCT will be designed.

Eligibility Criteria: Patients from 18 to 65 years of age with AML in first or second morphologic complete remission with an HLA-identical sibling donor are eligible. The donor must be healthy and willing to undergo G-CSF-based stem cell mobilization. Patients must have a Karnofsky performance status $\geq$ 70%. Patients must be in good clinical condition without coexisting medical problems that would significantly increase the risk of the transplant procedure. Patients must be free of active infections at the time of transplantation.

Treatment Description: Following screening and enrollment, the donor will receive mobilization therapy with daily G-CSF at a dose of 16 µg/kg/day subcutaneously. Leukapheresis will be performed on a continuous flow cell separator according to institutional standards commencing on Day 5 of G-CSF treatment. Daily leukapheresis of the donor with subsequent $CD34^+$ cell selection using the Miltenyi CliniMACS device will continue until a post-selection target of > 5.0×10^6 $CD34^+$ cells /kg recipient body weight and < 1.0×10^5 $CD3^+$ cells /kg recipient body weight is reached following at least two but not more than three leukapheresis procedures. There is no limit to the number of $CD34^+$ progenitors that can be administered.

The conditioning regimen will include total body radiation (TBI), thiotepa, cyclophosphamide, and rabbit antithymocyte globulin (ATG, thymoglobulin). The $CD34^+$ selected cells will be infused within 48-72 hours after the last dose of the conditioning regimen. Hyperfractionated TBI is administered at a dose rate of 8-20 cGy/minute. Doses of 125 cGy/fraction are administered at 4-hour intervals three times/day for a total of 11 doses (1375 cGy) over 4 days (Day -9, -8, -7, and -6).

Thiotepa will be administered at a dose of 5mg/kg/day IV for two consecutive days (Day -5, and -4). **Cyclophosphamide** will be administered at a dose of 60 mg/kg/day IV for two consecutive days (Day -3, and -2). **Rabbit Antithymocyte Globulin** (Thymoglobulin, Genzyme) will be administered as a single intravenous dose on Day -4 at 2.5mg/kg over 6-8 hours.

No additional GVHD prophylaxis will be administered. Due to stringent T cell depletion, no significant GVHD is anticipated. Should GVHD occur, the appropriate treatment schedule and dose will be initiated.

Accrual Objective: The sample size is 45 patients for this trial.

Accrual Period: It is anticipated that the accrual will last one year.

Study Duration: Patients will be followed for at least two years following transplantation.

Schema of Conditioning Regimen

-9	-8	-7	-6	-5	-4	-3	-2	-1	0	+1
TBI	TBI	TBI	TBI							
				THIO	THIO					
					rATG	CTX	CTX			
									$CD34^+$ PBSC Transplant	$CD34^+$ PBSC Transplant
Donor Mobiliza-tion				X	X	X	X	X Begin Leu-kapheresis	X Continue Leu-kapheresis	X Continue Leu-kapheresis, if necessary

TBI=total body irradiation; THIO=Thiotepa; rATG=rabbit antithymocyte globulin;
CTX=cyclophosphamide

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CHAPTER 1

1. BACKGROUND AND RATIONALE

1.1 Background

Allogeneic hematopoietic cell transplantation is an accepted therapy for acute myelogenous leukemia (AML). Transplants of unmodified HLA-matched related bone marrow or peripheral blood stem cells following conditioning with total body irradiation (TBI) and cyclophosphamide or VP-16 or busulfan and cyclophosphamide have led to sustained disease-free survival (DFS) rates of 45-60% for adults transplanted in first complete remission (CR1) and 40-53% for patients transplanted in second complete remission (CR2).[1] In several single center and multicenter cooperative group prospective trials comparing HLA-matched allogeneic transplants to chemotherapy in the treatment of AML in CR1, DFS rates for the transplant arm were almost invariably superior; however, these advantages were statistically significant in only a minority of the cooperative group studies conducted.[2, 3, 4, 5] In each study, the risk of relapse was significantly lower for patients receiving allogeneic transplants. However, this advantage was counterbalanced by transplant-related mortality, principally reflecting infections complicating graft-versus-host disease (GVHD) and its treatment.

The early morbidity and mortality associated with acute GVHD is a major factor limiting the success of transplantation as is the long-term morbidity associated with chronic GVHD. The risk of acute GVHD following allogeneic bone marrow transplantation (BMT) from HLA-matched siblings is 20-60% despite the use of immunosuppressive agents like cyclosporine A (CSA), tacrolimus (FK506), methotrexate (MTX), antithymocyte globulin (ATG) and corticosteroids, alone or in combination.[6, 7, 8, 9, 10, 11] Grades II-IV (moderate to severe) acute GVHD are associated with an increased risk of transplant-related mortality.[12, 13, 14] Mortality rates among patients who develop GVHD can be as high as 75% when that disease is unresponsive to therapy.[15] Recently, Forman et al. conducted a randomized trial comparing the use of CSA and corticosteroids versus the triple combination of CSA, MTX and corticosteroids for the prevention of GVHD in 147 patients with acute or chronic leukemia (personal communication). Preliminary results of this trial indicated that the three drug regimen decreased the incidence of acute GVHD to 11% (as compared to 22% in the patients receiving the two drug regimen), which is the lowest risk of GVHD reported thus far using non-T cell depleted grafts. There was no increase in leukemic relapse but there was a significant increase in the incidence of post-transplant fungal infections.

In summary, multi-agent pharmacological GVHD prophylaxis regimens appear capable of reducing the incidence of moderate to severe acute GVHD, but this has not translated into a significant improvement in overall survival due to increased toxicity or greater risk of opportunistic infection.[6, 10, 11]

Approximately one third of patients undergoing unmodified allogeneic BMT from HLA-matched siblings will develop chronic GVHD.[10, 11, 16, 17, 18] This complication can be extensive affecting

the skin, liver, lungs, gastrointestinal tract, mucous membranes, salivary and lacrimal glands.[19] In addition, this disorder results in impaired immune reconstitution thereby predisposing patients to infections. Chronic GVHD is therefore a significant cause of late post-transplant morbidity and morbidity and can result in reduced quality of life. Unfortunately, the use of immunosuppressive agents for prevention of acute GVHD has not affected the incidence of chronic GVHD.[10, 11]

1.2 Rationale

Immunosuppressive agents, therefore, are only partially effective in reducing the incidence and severity of acute GVHD, and have to date failed to decrease the incidence of chronic GVHD. Despite immunosuppressive therapy, engrafted donor T-lymphocytes derived from the marrow graft can respond to alloantigens on the cell surface of host cells, initiating the pathologic process leading to GVHD.[20, 21, 22]

Over the past twenty years, several techniques for T cell depletion (TCD) of donor grafts were introduced and evaluated for their capacity to prevent acute and chronic GVHD. These include techniques involving physical adsorption of T cells to protein ligands such as lectins, elutriation, and immunoadsorption or immunodeletion with T cell or lymphocyte-specific monoclonal antibodies. Comparative analyses have demonstrated that these techniques vary widely in their capacity to deplete T cells (1.5-3.2 $\log_{10}$ depletion). The relationship between the T cell dose transplanted and the risk of acute GVHD is complex and varies depending upon the degree of MHC-compatibility, graft source (bone marrow versus peripheral blood), type of GVHD prophylaxis (pharmacological versus T cell depletion), method of T cell depletion, conditioning regimen, use of in vivo depleting antibodies, as well as patient and donor characteristics including underlying disease, age, and gender. One study in more than 200 patients suggested that the threshold dose of T cells required to induce grade II-IV acute GVHD after HLA-matched sibling transplants is 10^5 clonable T cells/kg.[23] A 2.8-3.0 $\log_{10}$ reduction in marrow grafts or a 4.0 – 4.5 $\log_{10}$ reduction in peripheral blood stem cell (PBSC) grafts is required if this threshold is not to be exceeded. This likely explains the variable reductions in GVHD and inconsistent reductions in chronic GVHD observed with several techniques despite the concomitant use of CSA and MTX prophylaxis.

Certain negative selection approaches are effective in reducing or preventing acute GVHD in both HLA-matched and HLA-disparate transplant recipients without co-administration of CSA and/or MTX. These include: 1) T cell depletion by soybean lectin agglutination and E-rosette depletion (SBA), an approach reported by Memorial Sloan-Kettering Cancer Center (MSKCC) and the University of Perugia to be associated with a 0-5% incidence of acute GVHD in HLA-matched transplant recipients and a 0-8% incidence in HLA haplotype disparate transplant recipients; and, 2) depletion with the anti-CD6 monoclonal antibody reported to be associated with incidences of grade II-IV acute GVHD of 20% and 42% in HLA-matched related and unrelated transplant recipients, respectively.[24, 25, 26] These techniques have been primarily employed for the depletion of bone marrow allografts. Several recent randomized trials have compared bone marrow to peripheral blood stem cells (PBSC) as HLA-identical allografts sources.[27, 28, 29, 30, 31, 32, 33] All demonstrate improved kinetics of hematopoietic engraftment following PBSC and, in some, a survival advantage associated with the use of PBSC. However,

the risk of GVHD following PBSC transplantation is either equivalent to or greater than following bone marrow transplantation and GVHD following PBSC may be more difficult to treat. Therefore, strategies designed to deplete PBSC allografts of T cells in order to mitigate the risk of GVHD while retaining the favorable engraftment characteristics are of considerable interest. Unfortunately, there are currently few published data available detailing the use of T cell depletion following PBSC allografting. With G-CSF mobilized PBSC grafts T cell depleted with an immunoadsorption column, Urbano-Ispizua found that $CD3^+$ cell doses > $5x10^4$/kg were associated with a higher risk of acute GVHD.[34] However, Cornelisson and colleagues performed a similar study transplanting G-CSF mobilized HLA-matched allografts T cell depleted by immunoadsorption or immunoaffinity techniques and demonstrated that doses > 2.0 x 10^5 $CD3^+$ cells/kg increased the risk of GVHD.[35] The most compelling data are derived from studies in recipients of HLA-haploidentical related allografts. Aversa initially reported results with haploidentical PBSC allografts T cell depleted using an immunoadsorption column followed by soybean lectin agglutination. Allografts containing a median $CD3^+$ dose of 2.0 x 10^5/kg were associated with an 18% incidence of grades 2-4 acute GVHD.[36] Subsequently, the same group has used an immunoaffinity system (CliniMACS device) to T cell deplete G-CSF mobilized haploidentical allografts. This process results in a 4-5 log $CD3^+$ depletion and a median $CD3^+$ dose of 0.2 x 10^5/kg.[36] Despite the transplantation of haploidentical grafts without any pharmacological GVHD prophylaxis, the incidence of acute GVHD was less than 10%. Similar results are reported in pediatric transplant recipients by Handgretinger et al.[37] Among 56 HLA-matched related donor transplant recipients reported from Essen and Ulm, transplants of $CD34^+$ PBSC fractions isolated by the CliniMACS system were associated with a 0-4% incidence of grade II-IV acute GVHD.[38] However, when these patients were intentionally infused with 10^5 T cells/kg post-transplant, 14% developed grades II-IV acute GVHD. Together, the data suggest that if no pharmacological GVHD prophylaxis is used, $CD3^+$ cell doses should be kept below $1x10^5$/kg in order to reliably reduce the risk of moderate to severe acute GVHD.

In early series, T cell depleted transplants were associated with a 5-15% incidence of graft rejection.[39] Studies conducted at MSKCC demonstrated that these graft failures were principally caused by residual host T-lymphocytes that regenerate early after transplantation. These recipient T cells are able to reject donor hematopoietic cells through their cytotoxic interactions with major class I or class II HLA alloantigens in recipients of HLA-disparate transplants, or with minor alloantigens presented by HLA class I determinants in recipients of HLA-matched grafts. A sequence of clinical trials conducted at MSKCC over the last eight years has led to the identification of a conditioning regimen combining hyperfractionated TBI, thiotepa and cyclophosphamide (CTX) with ATG that reduces the incidence of graft failures following T cell depleted unrelated and 1-2 allele disparate marrow grafts to < 8%.[25, 40] These rates of graft failure are comparable to those observed after unmodified marrow transplants.[11]

Most of the series described above utilize cytoreductive regimens combining single dose TBI (750 cGy) or fractionated TBI (1200-1500 cGy) coupled with thiotepa 10mg/kg, either CTX or fludarabine (FLU), and ATG to prevent graft rejection. In the series reported from MSKCC, Perugia and Essen, the incidence of graft failure after HLA-matched transplants in adults is only 0-2% and 2% after HLA haplotype disparate transplants.

Persistent immune dysfunction is a frequent complication of BMT and may result in late mortality related to opportunistic infections, particularly in patients with chronic GVHD.[41, 42, 43, 44, 45, 46, 47, 48] A functioning thymus is crucial for effective immune reconstitution after BMT, particularly with respect to reemergence of naive CD4 cells.[47, 48, 49, 50, 51] The process of positive and negative selection of thymocytes within the thymus is necessary to produce a T cell population with a diverse T cell receptor (TCR) repertoire. Although it is apparent that thymic tissue functions in advanced age, a clear inverse relationship exists between thymic output and age.[40, 49, 52] Thus, thymic damage due to advancing age, prior chemotherapy, and the effects of radiation thwart immune reconstitution in older individuals.[53] Numerous studies document delayed recovery in adult compare to pediatric BMT recipients, even in the absence of GVHD, particularly in recipients of T cell depleted, mismatched- or unrelated-donor transplants.[47,40, 52, 54, 55] Active GVHD further damages the thymic and lymphoid micro-environments and greatly hinders T and B cell recovery.[51, 56, 57, 58] Thymic independent reconstitution of the T cell compartment occurs through the expansion of T cells infused along with the graft, but produces a T cell population with more limited TCR diversity.[50, 54]

Peripheral blood T-lymphocyte counts in adults do not normalize until 3 to 12 months after BMT.[42] Functional T cell recovery may be even more protracted, particularly after TCD BMT.[59, 60, 61] In vitro studies of T cells after BMT demonstrate reduced proliferative responses to mitogenic stimuli, abnormal cytotoxic T cell effector function, and impaired ability to collaborate with B cells in immunoglobulin synthesis.[42, 62, 63, 64] Functional recovery of T cells appears to be impaired after TCD BMT. In recipients of TCD marrow, the proliferative response of peripheral blood mononuclear cells to exogenous IL-2 stimulation remains abnormal for up to 6 months, compared to only 1 month for recipients of non-TCD BMT.[65] Similarly, the proliferative response of T cells to mitogenic stimulation can be impaired for over 18 months after TCD BMT.[65] T lymphocytes from recipients of TCD BMT also have significantly restricted variability in their TCR repertoires.[46, 66]

Immunophenotyping studies following TCD BMT demonstrate that NK cells are the first lymphoid subset to emerge, usually within 2 to 3 weeks after transplantation, followed by B cells (3-6 months) and T cells (3-12 months). Total lymphocyte numbers are usually higher early after BMT in recipients of non-TCD compared to TCD grafts. $CD8^+$ T cells typically reconstitute the T cell compartment first, and most TCD BMT patients have a deficit in $CD4^+$ cells, with an inverted $CD4^+$ to $CD8^+$ ratio for up to 2 years.[52, 67] The number of $CD4^+$ cells normalizes at 7 to 9 months after non-TCD BMT, but this process is delayed after TCD BMT.[68]

The delayed reconstitution of $CD4^+$ cells and impaired recovery of TCR diversity may predispose recipients of TCD BMT to opportunistic infections for months to years after transplantation. Although there is little reported evidence to suggest an increased risk of bacterial or fungal infections after TCD transplantation, several demonstrate a higher probability of reactivation of viruses such as CMV,[69, 70, 71] and EBV, and increased risk for EBV-associated B-cell lymphoproliferative disorders (EBV-LPDs) after TCD transplantation.[72, 73] Therefore, recipients of TCD allografts require ongoing surveillance for opportunistic viral pathogens.

1.3 Overview

Despite increased risks of infection, development of effective TCD techniques for prevention of GVHD and tolerable modifications of regimens for pre-transplant cytoreduction that secure consistent engraftment offer the potential for significant decreases in transplant-related mortality. Furthermore, the use of TCD transplants in the treatment of patients with AML is not associated with substantial increases in the incidence of relapse. Several single center trials indicate highly encouraging long-term results, particularly for patients with AML in CR1 or CR2 (Table 1.3.1). Although the number of cases in each single center series is limited, the consistency of the results suggests that the use of an effective technique for T cell depletion together with an adequate cytoreductive regimen might yield transplant results superior to those achieved with unmodified grafts.

TABLE 1.3.1: HLA-MATCHED SIBLING TCD TRANSPLANTS FOR AML

			AML 1st CR	***AML 2nd CR***
Center	**Cytoreduction**	**TCD Transplant**	**N OS DFS REL**	**N OS DFS REL**
MSKCC[25]	TBI/THIO/ CTX/ATG	SBA$^-$E$^-$BMT	44 73% 73% 7%	14 64% 64% 8%
PERUGIA[24]	TBI/THIO/ CTX/ATG	SBA$^-$E$^-$BMT	15 73% 73% 6%	10 60% 60% 20%
DFCC[26]	TBI/CTX	CD[6] Depleted BMT	28 70% 70% 31%	
Hammersmith/ Ulm	TBI/CTX	CAMPATH *in vitro* and *in vivo*	70 62% 60% 30%	

Of the currently available techniques providing a level of T cell depletion sufficient to prevent both acute and chronic GVHD, the CliniMACS System presents several advantages:

1) The single step positive selection of CD34$^+$ precursors from PBSC by the CliniMACS system alone provides the requisite level of T cell depletion required to prevent GVHD after both HLA-matched and HLA-disparate transplants.

2) The CliniMACS system has a registered master file at the Food and Drug Administration (FDA), and several Investigational Device Exemptions (IDEs) have already been granted for trials of CliniMACS selected, HLA haplotype disparate CD34$^+$ PBSC in the treatment of malignant and genetic hematopoietic diseases. An additional IDE could be obtained on the basis of an approved protocol.

3) Many BMT CTN centers have the Miltenyi device and are familiar with its operation.

While clinical experience with the use of CliniMACS separated CD34$^+$ PBSC for HLA-matched transplants for AML in early remission is extremely limited, the doses of CD34$^+$ cells and CD3$^+$

T cells provided by these grafts are extensively documented. Using a cytoreductive regimen including TBI/thiotepa/ATG and either CTX or FLU, engraftment and durable hematopoietic reconstitution are achieved in 100% of HLA-matched transplants (N=56), and 95-100% of HLA-disparate transplants (N=140) using grafts processed with the CliniMACS system. Grades II-IV acute GVHD rates range from 0-4% for patients transplanted with CliniMACS $CD34^+$ selelcted PBSC alone (Table 1.3.2), with relapse rates for patients transplanted for AML in remission of 11-13%. Thus, this approach is appropriate for clinical evaluation.

TABLE 1.3.2: MILTENYI EXPERIENCE

CENTER	PTS[1]	DONOR	TCD	CYTOREDUCTION	N	ENG	GF	GVHD II-IV	cGVHD
PERUGIA	A	Haplo	$CD34^+$ selected	TBI/THIO/FLU/ATG	53	98%	1	4%	1%
LISBON[2]	A	Haplo	$CD34^+$ selected	THIO/MEL[3]/FLU/ATG	14	100%	0	5%	2%
ESSEN[2]	A	Matched	$CD34^+$ selected	TBI/THIO/CTX/ATG	32	100%	0	4%	14%
TUBINGEN	P	MUD.	$CD34^+$ selected	Bu[4] or TBI/CTX/VP-16 or THIO	30	100%	0	10%	7%
ULM	A	Matched	$CD34^+$ selected	Bu or TBI/CTXorTHIO, or ATG	24	95%	0-5%	0%	27%

1: ENG=Engrafted; P = Pediatric; A = Adult; Haplo=haploidentical; Matched=HLA-identical relative; MUD-HLA-matched unrelated
2: Add back T cells 95 or 120 days post-transplant, beginning at $1x10^5$/kg up to $3x10^7$/kg
3: Melphalan
4: Busulfan

It appears that the key to successful application of this approach involves the procurement of an allograft containing the requisite number of $CD34^+$ cells to ensure prompt and durable engraftment while simultaneously reducing the number of alloreactive $CD3^+$ cells to below a threshold likely to induce moderate to severe acute GVHD. These prerequisites are best fulfilled using cytokine mobilized PBSC.

1.4 Procurement of PBSC Allografts

At Washington University, HLA-matched donors receive a once daily subcutaneous injection of G-CSF at 10μg/kg/day. On Day 5, the donors undergo a leukapheresis procedure with a target blood volume of between 18-20 liters/session. The target $CD34^+$ cell dose is 4.0 x 10^6/kg recipient weight. The numbers of $CD34^+$ cells obtained following the first collection and in total are depicted in Table 1.4. The mean number of leukapheresis procedures performed per donor is 1.2+/-0.2.

TABLE 1.4: $CD34^+$ DOSES OBTAINED FOLLOWING LEUKAPHERESIS OF HLA-IDENTICAL RELATED DONOR (N=109)

Percentile	1st Collection	Total Collections
50th	8.5 x 10^6/kg	9.2 x10^6/kg
80th	3.85 x10^6/kg	4.3 x10^6/kg
90th	2.3 x 10^6/kg	3.6 x10^6/kg

For this study, a dose of 16μg/kg/day G-CSF for donor mobilization was chosen based on data from Bensinger et al demonstrating the dose is well tolerated and is associated with an optimum $CD34^+$ cell mobilization.[28] Given an expected average $CD34^+$ cell yield of 50% following $CD34^+$ selection with the CliniMACS device, the data from both Washington University and Seattle suggest that at least 90% of donors on this trial should mobilize a sufficient number of $CD34^+$ cells to achieve the minimum targeted post selection $CD34^+$ cell dose of 2.0 x 10^6/kg following up to three leukapheresis procedures.

In this single-arm Phase II trial, we propose to assess if, and to what degree, the anticipated endpoints for the T cell depleted transplant arm of a planned prospective randomized trial comparing T cell depleted and unmodified marrow grafts applied to the treatment of AML in CR1 or CR2 are likely to be achieved in a multicenter study conducted by the BMT CTN. This single-arm trial will evaluate the likelihood of achieving the following targeted clinical endpoints: 6 month DFS > 75%, 2 year DFS for CR1 patients > 70%, 2 year DFS for C2 patients > 60%, graft failure rate < 5%, incidence of grade II-IV acute GVHD < 15%, and one-year incidence of transplant-related mortality of < 30%. It will also evaluate to what degree targeted doses of $CD34^+$ progenitors and $CD3^+$ T cells can be achieved following fractionation of PBSC grafts with the CliniMACS system.

CHAPTER 2

2. STUDY DESIGN

2.1 Study Overview

This single-arm Phase II trial is designed to assess the efficacy of HLA-identical sibling transplantation using PBSC grafts processed by the CliniMACS system and employing an intensive pre-transplant cytoreductive regimen. Efficacy will be defined by 6 month disease-free survival (DFS). Secondary endpoints include the rates of engraftment, acute and chronic GVHD, transplant-related mortality, 2 year DFS, overall survival and achievement of targeted $CD34^+$ and $CD3^+$ cell doses.

2.2 Hypothesis and Study Objectives

2.2.1 Hypothesis

Based on published results of non-TCD transplants from HLA-matched siblings applied to patients with AML in CR1 or CR2, the following would represent a significant improvement in results: 6 month DFS rate of > 75%, transplant-related mortality rate at 1 year < 30% and a DFS rate at two years > 70% for patients transplanted in CR1 and > 60% for patients transplanted in CR2. We hypothesize that these results will be achieved using the conditioning regimen and processed graft described below, specifically that 6 month DFS will be > 75% where events are death or relapse.

2.2.2 Study Objectives

The specific aims of this single-arm Phase II trial will be:

1. To assess DFS at 6 months post-transplant.
2. To assess the incidence of durable hematopoietic engraftment.
3. To assess the incidence and severity of acute and chronic GVHD.
4. To estimate the probability of survival and DFS at two years post-transplant.
5. To assess the incidence of transplant-related mortality.
6. To determine the proportion of patients receiving optimal $CD34^+$ ($> 5 \times 10^6$/kg) and $CD3^+$ ($< 1 \times 10^5$/kg) cell doses; the proportion of patients receiving $CD34^+$ cell doses $< 2 \times 10^6$/kg; and, the proportion of patients receiving $CD3^+$ cell doses $> 1 \times 10^5$/kg.

2.3 Patient Eligibility

Patients must meet specified eligibility criteria to be registered on the study.

2.3.1 Patient Inclusion Criteria

1. Patients with AML, with or without prior history of myelodysplastic syndrome, based on the World Health Organization criteria (see Appendix A) at the following stages:
   -- First morphologic CR
   -- Second morphologic CR

   The definition of morphologic CR is provided in Section 3.1. Patients with therapy-related AML are also eligible.

2. Patients with prior history of central nervous system (CNS) involvement are eligible provided there is no evidence of active CNS leukemia during the pre-transplant evaluation (no evidence of leukemic blasts in cerebrospinal fluid).
3. First or second CR was achieved after no more than two cycles of induction (or re-induction for patients in second CR) chemotherapy.
4. No more than six months can elapse from documentation of CR to transplant for patients in first CR and no more than 3 months can elapse from documentation of CR to transplant for patients in second CR.
5. Patients must be at least 18 years of age and less than or equal to 65 years of age.
6. Patients must have a 6/6 HLA antigen (A, B, DRB1)-compatible sibling donor for entry on this protocol. The match may be determined at serologic level for HLA-A and HLA-B loci. DRB1 must be matched at least at low-resolution using DNA typing techniques. HLA-C will be typed at the serologic level, but not included in the match algorithm.
7. Patients must have a Karnofsky performance status $\geq$ 70%.
8. Patients must have a life expectancy that is greater than eight weeks.
9. DLCO must be > 40% (corrected for hemoglobin) with no symptomatic pulmonary disease.
10. LVEF by MUGA or echocardiogram must be $\geq$ 40%.
11. Patient must have serum creatinine < 2 mg/dL, bilirubin < 2 mg/dL, AST and ALT $\leq$ 3 x upper limit of normal at time of enrollment.
12. Each patient and donor must be willing to participate as a research subject and must sign an informed consent form.

2.3.2 Patient Exclusion Criteria

1. Patients with M3-AML (acute promyelocytic leukemia) in first CR.
2. Patients with acute leukemia following blast transformation of prior chronic myelogenous leukemia (CML) or other myeloproliferative disease.
3. Patients with M4Eo-AML with inv 16 in first CR.
4. Patients with AML with t(8;21) in first CR.

5. Participation in other clinical trials that involve investigational drugs or devices except with permission from the Medical Monitor.
6. Evidence of active Hepatitis B or C infection or evidence of cirrhosis.
7. Patients who are HIV positive.
8. Patients with uncontrolled diabetes mellitus.
9. Patients with a proven or probable invasive fungal infection must have their infection controlled. Patients may be on prophylactic anti-fungal agents, but are not permitted to be on anti-fungal agents for therapeutic purposes (i.e., active treatment for disease).
10. Patients with an uncontrolled viral or bacterial infection (currently taking medication without clinical improvement).
11. Documented allergy to iron dextran or murine proteins.
12. Women who are pregnant (positive serum or urine β-HCG) or breastfeeding. Women of childbearing age must avoid becoming pregnant while on the study.
13. Patients having a prior autologous or allogeneic HSCT.

2.4 Initial Donor Eligibility Criteria

2.4.1 Donor Inclusion Criteria

1. The donor must be examined and have specific tests performed according to existing institutional guidelines and meet institutional guidelines for candidacy as a donor.
2. Donor age must be < 75 years and weight greater than 25 kg.
3. Donor must be seronegative for HIV Ag, HIV 1+2 Ab, HTLV I/II Ab, HBsAg, HBcAb (IgM and IgG), HCV Ab, RPR for syphilis within 30 days of apheresis collection.
4. The donor, or legal guardian greater than 18 years of age, must have been informed of the investigational nature of this study and have signed an informed consent form. Donors < 18 years of age must provide assent to participate in the study.
5. Donor must be agreeable to second donation of PBSC in the event of graft failure or EBV-related lymphoproliferative disease, if necessary.
6. Donor must be agreeable to undergoing general anesthesia and bone marrow donation, if necessary.
7. Donor must have adequate peripheral venous catheter access for leukapheresis or must agree to placement of a central catheter.

2.4.2 Donor Exclusion Criteria

1. Evidence of active infection (including urinary tract infection, or upper respiratory tract infection) or viral hepatitis exposure (on screening), unless only HB_S Ab+ and HBV DNA negative.

2. Medical or physical reason which makes the donor unlikely to tolerate or cooperate with growth factor therapy and leukapheresis.
3. Factors which place the donor at increased risk for complications from leukapheresis or G-CSF therapy (e.g., autoimmune disease, sickle cell trait, symptomatic coronary artery disease requiring therapy).
4. Pregnancy (positive serum or urine β-HCG) or breastfeeding. Women of childbearing age must avoid becoming pregnant while on the study.

2.5 Study Treatments

2.5.1 Mobilization of Donor

Following screening and enrollment, the donor will receive mobilization therapy with once daily G-CSF. Mobilization will begin on Day -5 of the conditioning regimen at a G-CSF dose of 10 μg/kg/day actual body weight subcutaneously (rounded off to a multiple of the nearest vial size of either 300 or 480 μg). Based on the volume, the dose may be split into 2 or 3 injection sites. The mobilization phase starts on the first day of administration of G-CSF and continues until the final day of leukapheresis.

2.5.2 Progenitor Cell Collection and Processing

2.5.2.1 Leukapheresis

Leukapheresis will be performed on a continuous flow cell separator according to institutional standards and will commence on the morning of the fifth day of G-CSF treatment. The anti-coagulant used for the procedure will be acid citrate dextrose (ACD). No additional anti-coagulants or additives (heparin, etc.) should be added beyond those normally used during leukapheresis. The volume of blood processed per leukapheresis session should be approximately three to four times total blood volume as tolerated by the donor. A unique identification and labeling system shall be used to track the leukapheresis product from collection to infusion according to AABB and FACT guidelines.

The target allograft cell doses following processing on the CliniMACS device are a $CD34^+$ cell count of > 5.0 x 10^6/kg recipient weight together with a maximum $CD3^+$ cell dose of < 1.0 x 10^5/kg recipient weight. Additionally, using the algorithm described in Table 2.5.2.3 below, a minimum $CD34^+$ dose of 2.0 x 10^6/kg will be targeted. If this minimum target CD34+ cell dose is not achieved after three leukapheresis procedures and CD34 selection, the protocol requires an absolute minimum $CD34^+$ dose of 1.0 x 10^6/kg in order for the patient to be evaluable for all study endpoints. If the post $CD34^+$ selection allograft content is < 1.0 x 10^6 $CD34^+$ cells/kg recipient weight after two leukapheresis procedures, the transplant center will perform a third leukapheresis collection without $CD34^+$ selection in order to ensure that an adequate $CD34^+$ cell dose is transplanted. Since this graft will contain a high number of $CD3^+$ cells, such patients must also receive pharmacological GVHD prophylaxis per institutional standards (refer to Table 2.5.2.3). If after a third leukapheresis and $CD34^+$ selection the allograft contains a total $CD34^+$

cell dose between 1-2 x 10^6/kg but the $CD3^+$ dose is < 1.0 x 10^5/kg, the patient will continue to be considered evaluable for all study endpoints.

During mobilization and leukapheresis, institutional standards for donor supportive care should be maintained.

2.5.2.2 $CD34^+$ selection with CliniMACS device

$CD34^+$ cell selection will be performed according to procedures given in the CliniMACS Users Operating Manual and institutional Standard Operating Procedures (SOPs) in place and validated at the study sites. Products will be received into the cell processing laboratories and will be either processed that day, or stored overnight. Products stored overnight must be processed the following day. At receipt the product will be accessioned and assigned a unique product identifier if not already assigned during collection. The product will be inspected at the time of receipt and the label information regarding donor name, medical record number (or other identifier) and ABO and Rh group will be compared for consistency to the information in the laboratory chart record and physicians order form. The product will be sampled for total nucleated cell count (TNC), and if processing is to be performed on the day of receipt, additional sample will be obtained for starting CD34+ cell and starting T cell content and product sterility. Products may be stored overnight for one of two reasons, 1) late arrival preventing selection and infusion the same day, or 2) low TNC content that would permit pooling of two collections for a single column selection. If the product is to be stored overnight, the cells will be diluted to < 2.0 x 10^8 cells/mL using either autologous plasma or the CliniMACS PBS/EDTA-1.0% Human Serum Albumin (HSA) (CliniMACS buffer) and stored in a monitored refrigerator at either 1-6^{o}C or 2-8^{o}C depending upon institutional policy. Products that are stored overnight will be sampled the following day, prior to processing, for TNC, starting CD34+ cell and starting T cell content, and for sterility.

Cell processing personnel will receive training by Miltenyi on the CliniMACS system prior to initiation of clinical product selection. The site will provide documentation to Miltenyi on competency in the processing procedures including the results of validation runs on the CliniMACS System.

2.5.2.3 Analysis of allograft

Samples will be taken from each leukapheresis product pre- and post-$CD34^+$ selection and characterized as follows:

- Graft Evaluation (all of these tests are standard of care)
  - Gram stain (done locally) post-selection on each leukapheresis product
  - Total nucleated cell count (done locally) pre- and post-selection on each leukapheresis product
  - Endotoxin testing post-selection on each leukapheresis product, done locally or sent to an authorized lab

- Flow cytometric analysis for $CD34^+$ cells pre- and post-selection done locally using validated SOPs
- Flow cytometric analysis for $CD3^+$ cells pre- and post-selection and log depletion on each leukapheresis product done locally using validated SOPs
- Viability testing (7-AAD method) post-selection
- 14 day sterility cultures post-selection on each leukapheresis product

- Criteria for release of product
  - Viability ≥ 70% after selection
  - Negative gram stain
  - $CD34^+$ cell count of product

As noted above, the target optimal allograft cell doses following processing on the CliniMACS device include both a $CD34^+$ cell count of > 5.0 x 10^6/kg recipient weight and a $CD3^+$ cell dose of < 1.0 x 10^5/kg recipient weight. The targeted minimum $CD34^+$ cell dose following $CD34^+$ selection is 2.0 x 10^6/kg. If after two leukapheresis collections followed by $CD34^+$ selection the total allograft contains 2-5x10^6 $CD34^+$ cells/kg and < 1.0 x10^5 $CD3^+$ cells/kg, a total of three leukapheresis collections followed by $CD34^+$ selection are allowed as long as the total $CD3^+$ cell dose transplanted does not exceed 1.0 x 10^5/kg. That is, a graft containing 2-5x10^6 $CD34^+$ cells/kg and < 1.0 x10^5 $CD3^+$ cells/kg is acceptable whereas a graft containing 5x10^6 $CD34^+$ cells/kg but > 1.0 x10^5 $CD3^+$ cells/kg is not. A major goal is to administer a $CD34^+$ cell dose> 5x10^6/kg while limiting the $CD3^+$ cell dose to < 1.0 x10^5/kg but as long as a minimum of 1x10^6/kg $CD34^+$ cells are infused, the $CD3^+$ cell dose should be kept to < 1.0 x10^5/kg. It may be possible to give only a proportion of the $CD34^+$ selected product in order to maintain an adequate $CD34^+$ cell dose while limiting the $CD3^+$ cell dose to < 1.0 x10^5/kg.

It is also possible that doses of $CD34^+$ cells far exceeding 5 x 10^6/kg can be given without exceeding the maximum T cell dose of 1 x 10^5 $CD3^+$ cells/kg. High doses of $CD34^+$ cells in extensively T cell depleted transplants are postulated to hasten immune reconstitution, without altering the low risk of GVHD. Consequently, there is no upper limit for the dose of $CD34^+$ cells/kg as long as the dose of $CD3^+$ cells does not exceed 1 x 10^5/kg. Furthermore, a minimum of 2 leukaphereses should be obtained from each donor even if the first leukapheresis provides 5 x 10^6 $CD34^+$ cells/kg, if the anticipated cumulative $CD3^+$ cell dose is expected to be less than 1 x 10^5/kg recipient weight.

However, if the post $CD34^+$ selection allograft from the first leukapheresis product is ≥ 5 x 10^6/kg recipient weight and close to the maximum $CD3^+$ cell dose allowed (≥ 0.7 x 10^5/kg recipient weight), it will be left to the discretion of the center PI to determine whether the leukapheresis product from Day 2 of collection should be $CD34^+$ cell selected or cryopreserved without further processing. Regardless of the choice, it is imperative that the total transplanted CD3+ cell dose not exceed 1 x 10^5/kg recipient weight. If a decision is made to $CD34^+$ cell select the Day 2 leukapheresis product, that $CD34^+$ cell selected product should only be transplanted into the recipient if the total $CD3^+$ cell dose will be < 1 x 10^5/kg recipient weight. Otherwise, the selected product from Day 2 should be cryopreserved.

Decisions concerning the release of samples from the $CD34^+$ selected product will be based on $CD34^+$ and $CD3^+$ cell analysis done by the flow cytometry laboratory at the site. Table 2.5.2.3 outlines various scenarios that may be encountered after each $CD34^+$ cell selection and indicates appropriate steps for achieving the target allograft cell doses.

TABLE 2.5.2.3: GUIDELINES FOR TRANSPLANTATION OF ALLOGRAFT PRODUCT FOLLOWING CD34$^+$ SELECTION

LP Attempt	Total CD34$^+$ Dose Following LP[1]				Total CD3$^+$ Dose after LP[1]		Action				
	< 1.0	1.0-2.0	2.0-4.9	≥ 5.0	< 1.0	≥ 1.0	Administer Allograft	Proceed to next LP	LP Completed	Reduce CD3$^+$ dose to < 1.0; Maintain CD34$^+$ dose > 2.0	Begin GVHD Prophylaxis
1				X	X		X	X[2]			
1				X		X		X		X[3]	
1	X[4]	X[4]	X[4]		X		X	X			
1	X[4]	X[4]	X[4]			X		X		X[3]	
2				X	X		X		X		
2				X		X				X[5]	
2		X[4]	X[4]		X		X	X			
2		X[4]	X[4]			X				X[6]	
2	X				X[7]	X[7]	X	X[8]			X[9]
3				X	X		X		X		
3			X		X		X		X		
3				X		X				X[10]	
3			X			X				X[10]	
3		X			X		X		X		
3		X				X					X[11]
3	X[12]				X[7]	X[7]					

Notes for this table continue on the next page.

1. LP is abbreviation for leukapheresis. This number refers to CD34$^+$ (x10^6/kg) or CD3$^+$ (x10^5/kg) doses after CD34$^+$ selection. If after LP #2 or #3, this refers to total (pooled) CD34$^+$ and CD3$^+$ doses following all CD34$^+$ selections
2. All patients will have a minimum of two leukapheresis.
3. If unsuccessful in reducing CD3$^+$ cell dose to below 1x10^5/kg at any CD34$^+$ dose, hold allograft overnight and proceed to LP #2. If LP#2 results in a post selection CD34$^+$ count of ≥ 2.0 x 10^6/kg with CD3$^+$ dose < 10^5/kg, give that allograft, proceed to LP#3, and discard or cryopreserve graft from LP#1. The primary goal is to reduce the total CD3$^+$ cell dose to < 1x10^5/kg while maintaining the total CD34$^+$ cell dose > 2x10^6/kg. Contact one of the Protocol Chairs if clarification necessary

4. Refers to any one of these three possible $CD34^+$ doses (< 1.0-4.9)
5. If unsuccessful in reducing $CD3^+$ cell dose to below $1x10^5$/kg at any $CD34^+$ dose, begin GVHD prophylaxis and then administer allograft within 24 hours.
6. If able to reduce $CD3^+$ dose to < 10^5/kg while maintaining $CD34^+$ dose $\geq$ 2.0, proceed to LP#3. If unsuccessful in reducing $CD3^+$ cell dose to below $1x10^5$/kg at any $CD34^+$ dose, begin GVHD prophylaxis and then administer allograft within 24 hours.
7. Refers to either one of the two possible $CD3^+$ doses
8. Proceed to LP#3, but do not perform $CD34^+$ selection on product from LP#3.
9. If the $CD3^+$ dose is < 10^5/kg, administer product and then begin GVHD prophylaxis. If the $CD3^+$ dose is $\geq$ 10^5/kg, administer product from LP#2 24 hours after starting GVHD prophylaxis.
10. If unsuccessful in reducing $CD3^+$ cell dose to below $1x10^5$/kg at any $CD34^+$ dose, hold allograft if patient has already received a $CD34^+$ dose $\geq$ 2.0 x 10^6/kg and a $CD3^+$ dose < 10^5/kg. Otherwise, give allograft from LP#3 24 hours after starting GVHD prophylaxis.
11. Administer product from LP#3 24 hours after starting GVHD prophylaxis
12. Contact one of the Protocol Chairs to discuss options

2.5.3 Conditioning Regimen

The conditioning regimen will include TBI, thiotepa (THIO), cyclophosphamide (CTX), and rabbit ATG (thymoglobulin). The $CD34^+$ selected cells will be infused within 48-72 hours after the last dose of cyclophosphamide.

2.5.3.1 Radiation therapy

Hyperfractionated TBI is administered at a dose rate of < 20 cGy/minute. Doses of 125 cGy/fraction are administered at a minimum interval of 4 hours between fractions, three times/day for a total of 11 doses (1375 cGy) over 4 days (Day -9, -8, -7 and -6). Sequential doses are administered in an anterior/posterior or lateral orientation. The orientation of TBI chosen will be left to the discretion of the radiation oncology specialist at each center but should remain consistent at each institution throughout the duration of the trial. Full value lung blocks are not allowed. Compensators and lung blocks yielding a minimum of 800 cGy lung dose are allowed based on institutional practice. Depending on the method of lung shielding employed and institutional practice, the blocked areas should be boosted with high-energy electrons or be otherwise radiated so that the cumulative chest wall dose is approximately 1300 cGy, so as to insure that marrow sites in the ribs are adequately treated. The Memorial Sloan-Kettering techniques for chest wall boosting and hyperfractionated TBI are provided in Appendices E and F.

2.5.3.2 Thiotepa

Thiotepa will be administered at a dose of 5mg/kg/day (ideal body weight) IV for two consecutive days (Day -5 and -4).

2.5.3.3 Cyclophosphamide

Cyclophosphamide will be administered at a dose of 60 mg/kg/day (ideal or adjusted body weight) IV for two consecutive days (Day -3 and -2). The Day –2 dose should be given in the morning, preferably before 10AM. Cyclophosphamide will be dosed using adjusted body weight for patients > 100% of ideal body weight (IBW) (Section 2.5.3.8).

2.5.3.4 Rabbit antithymocyte globulin (thymoglobulin)

Rabbit ATG will be given as a single intravenous dose on Day -4 using rabbit antithymocyte globulin (Genzyme) at a dose of 2.5 mg/kg (actual body weight). If the patient has a history of allergy or intolerance to rabbit ATG, equine antithymocyte globulin at a dose of 30 mg/kg may be used. The dose of ATG will be administered over 6-8 hours. Methylprednisolone 1 mg/kg will be given as premedication. Additional medications to prevent or treat reactions will be administered as indicated according to institutional guidelines.

2.5.3.5 Schema of conditioning regimen

TABLE 2.5.3.5: SCHEMA OF CONDITIONING REGIMEN

-9	-8	-7	-6	-5	-4	-3	-2	-1	0	+1
TBI	TBI	TBI	TBI							
				THIO	THIO					
					rATG	CTX	CTX			
									$CD34^+$ PBSC Transplant	$CD34^+$ PBSC Transplant
Donor Mobiliza-tion				X	X	X	X	X Begin Leu-kapheresis	X Continue Leu-kapheresis	X Continue Leu-kapheresis, if necessary

2.5.3.6 Day of transplantation

Forty-eight to seventy-two hours after the patient has completed treatment with cyclophosphamide, the patient will receive the transplant of positively selected $CD34^+$, T cell depleted peripheral blood stem cells.

The total number of $CD3^+$ T cells in the $CD34^+$ selected PBSC graft should be $< 1.0 \times 10^5$/kg recipient weight. If not, GVHD prophylaxis, per institutional guidelines, should be started and the selected PBSC graft infused 24 hours later (see Table 2.5.2.3).

2.5.3.7 Graft-versus-host disease prophylaxis

No post-transplant GVHD prophylaxis will be administered as long as the graft administered meets the specified cell dose requirements (see Table 2.5.2.3). Due to stringent T cell depletion, no significant GVHD is anticipated. Should GVHD occur, appropriate treatment will be initiated (see Section 2.6.11).

2.5.3.8 Body weight formulas

1. Ideal Body Weight

Patients Over 18 Years
Males IBW = 50 kg + 2.3 kg/inch over 5 feet
Females IBW = 45.5 kg + 2.3 kg/inch over 5 feet
For patients less than 5 feet, subtract 2.3 kg/inch

Patients 1 to 18 Years of Age

Less than 60 inches
IBW = (ht^2 x 1.65)/1000 where ht = cm, IBW = kg

More than 60 inches
Males IBW = 39.0 + [2.27 x (ht - 60)] where ht = inches, IBW = kg
Females IBW = 42.2 + [2.27 x (ht - 60)] where ht = inches, IBW = kg

2. For patients who weigh more than 100% of their IBW, dosing of thiotepa and cyclophosphamide should be based on the adjusted ideal body weight (AIBW).

Adjusted Ideal Body Weight (AIBW) Formula:

AIBW = IBW + [(0.25) x (actual body weight – IBW)]

3. For patients who weigh less than 100% of their IBW, dosing of thiotepa and cyclophosphamide should be based on actual body weight (ABW).

2.6 Supportive Care

Institutional standards for general supportive care after transplantation should be maintained and should include antimicrobial agents, nutritional support and blood product support as necessary. Supportive care parameters standardized for this protocol are detailed below.

2.6.1 Venous Access

Recipients will have appropriate long-term central venous access placed, per institutional standard practice, prior to beginning the conditioning regimen.

2.6.2 Blood Products

All blood products, except the infused $CD34^+$ PBSC, will be irradiated in accordance with institutional standards not to fall below 2500 cGy. Recipients who are CMV negative will receive either CMV negative blood products or leukocyte depleted blood products from study entry.

2.6.3 Anti-bacterial Prophylaxis

Prophylaxis against gram negative bacterial infection must be administered during the neutropenic phase. Suggested agents include ciprofloxacin, levofloxacin or gatifloxacin but the particular antibacterial agent used will be at the discretion of each institution.

Pneumocystis carinii prophylaxis will be administered per institutional guidelines until Day +100.

2.6.4 Anti-viral Prophylaxis

Epstein-Barr Virus (EBV) surveillance using a real time quantitative EBV DNA PCR plasma-based assay shall be performed weekly until Day +100 and then at least monthly until Day 180. Samples will be sent to the central laboratory at the University of Washington. Patients who develop EBV DNA levels of > 1000 copies/mL on any tests will receive 375 mg/m^2 of rituximab. Those patients that continue to have levels above 1000 copies/mL on subsequent testing will receive three additional weekly infusions of 375 mg/m^2 of rituximab. Patients who develop a fever of unknown origin to > 39^{o}C, lymphadenopathy, or hepatosplenomegaly, should undergo CT scanning of the chest and abdomen to rule out or stage EBV post-transplant lymphoproliferative disorder (PTLD). Tissue diagnosis that includes EBER and LMP-1 immunocytochemistry should be attempted. Other diagnostic or staging studies will be performed as clinically indicated. One of the Protocol Chairpersons should be consulted for advice regarding treatment of suspected EBV PTLD.

Cytomegalovirus (CMV) surveillance will be performed weekly until Day 100 and at least monthly through Day 180 using antigenemia or PCR-based assays per institutional guidelines. Patients with evidence of CMV viremia based on either assay will receive preemptive therapy per institutional guidelines.

Patients must receive acyclovir or valacyclovir through Day 180 as standard prophylaxis against HSV and VZV per institutional guidelines.

2.6.5 Anti-fungal Prophylaxis

Anti-fungal prophylaxis must be administered through at least Day 180 according to institutional guidelines.

2.6.6 Cytokine Administration

Growth factors will not be used following transplant unless absolute neutrophil cell count (ANC) recovery to 0.5x10^9/L has not occurred by Day +14. If by Day +14, the ANC is still < 0.5x10^9/L or if, after initial recovery, the ANC drops below 1 x 10^9/L, G-CSF may be given per institutional guidelines.

2.6.7 Febrile Neutropenia

Broad-spectrum antibiotics will be administered intravenously according to institutional guidelines.

2.6.8 Transfusions

Leukocyte-poor platelets will be administered when the platelet count is $< 10 \times 10^9$/L or per institutional guidelines. If the platelets are refractory as documented by no sequential platelet count rise within 1/2 hour post platelet transfusion, then administer platelets only for active bleeding. Platelets must be transfused upon any active bleeding. Packed red cell transfusions are recommended when hemoglobin is < 8 mg/dL or as clinically indicated.

2.6.9 Nutrition

A low microbial diet will be maintained while the recipient is in isolation. Parenteral nutrition will be initiated depending on the patient's needs.

2.6.10 Isolation

Recipients will be maintained in single occupancy rooms with protective isolation per institutional guidelines.

2.6.11 Management of Acute GVHD

Recipients who develop acute GVHD $\geq$ overall grade II will be treated per institutional guidelines. At a minimum, patients requiring treatment should receive methylprednisolone or another corticosteroid of equivalent potency at a methylprednisolone dose of 2 mg/kg/day.

2.6.12 Management of Graft Failure

Primary or secondary graft failure will be considered a treatment failure. Patients will be treated according to institutional guidelines at the investigator's discretion. Management of graft failure should be discussed with one of the Protocol Chairpersons. Second transplants can reconstitute hematopoiesis and lead to long-term survival with certain preparative therapy.

2.6.13 Management of Post-transplant Relapse

Patients who relapse (recurrence of AML after infusion of the selected $CD34^+$ cells) will be treated according to institutional guidelines.

2.7 Participant Risks

Recipients of allogeneic transplants are subject to risks from the conditioning regimen, processing and infusion of PBSC, and post-transplant therapy. These must be weighed against the risk of the malignancy for which the transplant is prescribed. Major risks specific to allogeneic PBSC transplantation on this protocol include the items below.

2.7.1 Potential Sensitization to Murine Proteins

Mouse protein antibodies are used in the CliniMACS processing procedures. If the recipient has a pre-existing allergy, he or she may be at risk for allergic reactions during infusion of the processed cells, although the residual amount of murine protein in the final product is very low (estimated maximum dose for a 50 kg patient would be 30 μg). To date, no allergic reactions are reported in patients receiving cells processed with the CliniMACS System. Epinephrine and antihistamines will be available at the recipient's bedside during the PBSC infusion.

2.7.2 PBSC Infusion

Symptoms may include changes in heart rate and/or rhythm, changes in blood pressure, fever, chills, sweats, nausea, vomiting, diarrhea, abdominal cramping, hemoglobinuria, acute renal failure, allergic reactions, respiratory dysfunction, or headache.

2.7.3 Infections

Transplantation puts the patient at higher risk for bacterial, viral, or fungal infections, which are potentially life threatening. These risks are potentially higher with TCD transplants. Prophylaxis will be initiated (see Section 2.6) and patients will be closely monitored for signs of infections and will receive early and appropriate treatment.

2.7.4 Microbial Contamination of PBSC

There is a potential that processing the leukapheresis product will inadvertently introduce microorganisms that could cause infection in the recipient after the cells are infused. All precautions to maintain sterility will be taken. Cultures of the leukapheresis product and the selected product will be obtained to monitor for contamination.

2.7.5 Graft Failure / Poor Marrow Function

T cell depletion of donor cells is associated with an increased incidence of graft failure in allogeneic transplant recipients. After allogeneic transplantation, the recipient's marrow function may be poor and leukopenia, anemia, or thrombocytopenia may result from many causes including GVHD, immunosuppressant drugs and other medications. Graft failure may result in death if not reversed. Second transplants can be administered with immunosuppressive therapy, including non-myeloablative conditioning regimens. One of the Protocol Chairs should be consulted for advice regarding treatment of suspected graft failure.

2.7.6 Graft-versus-host Disease

Acute or chronic GVHD may develop after allogeneic transplantation that can be disabling and can lead to death. GVHD is thought to be initiated by T cells contained in the PBSC graft. $CD34^+$ selection and $CD3^+$ depletion reduces the number of T cells in the PBSC but GVHD can occur after TCD transplants.

2.7.7 Veno-occlusive Disease (VOD) of the Liver

VOD is a manifestation of damage to the liver by the conditioning regimen that usually develops within two weeks after allogeneic transplant and is characterized by at least two of the following:

- Hyperbilirubinemia (total bilirubin > 2 mg/dL)
- Hepatomegaly or right upper quadrant pain, or
- Sudden weight gain (> 5% above baseline)

Recipients developing VOD will be monitored closely and will receive appropriate supportive care and careful fluid management. TCD is not expected to affect the risk of VOD.

2.7.8 End Organ Damage

End organ damage of all or any of the major organs, including the brain, may occur as a result of cumulative toxicity from anti-neoplastic therapy, reactions to other drugs, and as a result of destructive processes (e.g., infection, GVHD, etc.) and may have a fatal outcome. Toxicities may occur in any individual patient due to multiple events and cumulative effects that may involve any and all organs, including the brain. Brain damage can result in severe loss of cognitive or neurologic function. Data from previous studies do not suggest that the risk of end organ damage is appreciably affected by TCD or the preparative regimens to be used in this study.

2.7.9 Disease Relapse

Allogeneic transplantation using T cell depleted marrow has, in some cases, been associated with an increased incidence of tumor relapse, compared to recipients who receive unmanipulated donor cells.

2.7.10 Lymphoproliferative Syndrome

Recipients of TCD allogeneic grafts may have an increased risk of developing lymphoproliferative syndromes caused by EBV infection. This syndrome should be included in the differential diagnosis of recipients with unexplained symptoms such as fever, diarrhea, hepatomegaly or lymphadenopathy. Biopsy evaluation is required to make the diagnosis. EBV PTLD may rapidly progress and can be fatal if not treated. Management of suspected EBV PTLD should be discussed with one of the Protocol Chairpersons. EBV PTLD can be treated with rituximab and/or infusion of 10^6 T cells/kg from the donor. Rituximab has been shown to

induce regression in 50 - 70% of cases. Note: Rituximab does not enter the CNS and is not effective in treating CNS disease. Donor lymphocyte infusions may induce regression in > 90% of cases of EBV PTLD and are effective in CNS disease.

2.7.11 Death

There is an approximately 5-10% risk of treatment related mortality within the first month of transplant due to the risk of severe regimen related toxicity, hemorrhage, opportunistic infection, or other complications. It is not expected that the regimens to be used in this protocol will increase this risk.

2.8 Therapy Toxicities

All toxicities will be graded using the Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0.

2.8.1 Total Body Irradiation

1. Myelosuppression: Is the major dose-limiting toxicity.
2. Nausea and vomiting: Variable; most patients experience nausea and vomiting. This can be diminished with hydroxyzine, lorazepam, chlorpromazine or ondansetron. Strong sedatives should be avoided as they can cause excessive drowsiness and/or symptomatic orthostasis, which can prevent the delivery of TBI done in the standing position.
3. Parotitis: Some patients will experience symptomatic parotitis within the first 24 hours post radiation. This resolves spontaneously over several days.
4. Diarrhea: Most patients develop some diarrhea in the first week post radiation. This can be treated symptomatically.
5. Fever: Significant fever (greater than 38º C) may occur during the first day post radiation and can be treated symptomatically.
6. Erythema: A generalized erythema may occur in patients within 24 hours. It usually resolves over 2-3 days.
7. Hyperpigmentation: This complication occurs to some degree in most patients within 2-3 weeks following radiation. It takes several weeks to resolve.
8. Mucositis: Most patients will develop moderate to severe mucositis of the oral and gastrointestinal tract, which will be managed with aggressive nursing mouth care and prophylactic antifungal agents. Palifermin is not permitted to be used to manage mucositis.
9. Late effects: Late effects may occur with varying degree following TBI and include cataracts, growth failure, gonadal failure and sterility, hypothyroidism, and secondary malignancies.

2.8.2 Cyclophosphamide

1. Myelosuppression: Is the major dose-limiting toxicity.
2. Nausea and vomiting: Variable; symptomatically improved with traditional intravenous or oral antiemetics.
3. Water retention: Cyclophosphamide induced inappropriate secretion of ADH, usually manifested 4-8 hours after IV administration, necessitating frequent accurate (q1-2 hours) assessments of intake, urine output and urine specific gravity. This effect is counteracted by furosemide.
4. Cardiomyopathy: Cyclophosphamide may cause a severe, sometimes lethal cardiomyopathy. EKGs are monitored before and after cyclophosphamide infusions for evidence of toxicity. Congestive heart failure is managed with inotropic agents and diuretics. Cyclophosphamide is contraindicated in patients with significant cardiac disease.
5. Hemorrhagic cystitis: This is a serious, potentially life-threatening complication related to the interaction of cyclophosphamide metabolites and the bladder epithelium. Although hematuria is not uncommon at this dose level, serious hemorrhage can be avoided by maintaining a high urine volume and frequent voidings. Diuresis is maintained for 24 hours after each dose by parenteral infusions of 3,000 cc/m^2/day or more. Careful monitoring of serum and urine electrolytes is mandated. Furosemide may be required to insure this diuresis.
6. Alopecia: Transient and reversible.
7. Skin rash: This may develop 1-3 days post infusion and subsides thereafter.
8. Sterility: This is a likely complication after puberty.

2.8.3 Thiotepa

1. Myelosuppression: Is the major dose-limiting toxicity.
2. Nausea and vomiting: Variable; symptomatically improved with traditional intravenous or oral antiemetics.
3. Skin rash: Transient generalized skin erythema may happen following thiotepa. It involves mainly axillary and inguinal folds. It is usually erythematous with possibly blistering and scaling. Since Thiotepa is secreted in sweat, axilla and inguinal areas must be kept clean. Patients should shower twice daily during administration and for two days after completion. It develops 4-14 days after the first dose and may last up to 3 weeks. It may be followed by bronzing of the skin that may persist for months.
4. Central nervous system toxicity: Has occurred at higher doses (> 1,000 mg/m^2). It is unlikely to occur at this level. It is manifested by mild cognitive dysfunction, disorientation, confusion and irritability.

2.8.4 Rabbit Antithymocyte Globulin (Thymoglobulin)

The ATG to be used in this trial is a purified preparation of rabbit gamma globulin containing high concentrations of antibodies against human lymphocytes. The preparation may contain low levels of antibody that cross-react with human platelets, white cells or red cells. The potential side effects of ATG are:

1. Fever and chills: These are regularly observed, particularly during initial infusions of the rabbit globulin. They probably result from a breakdown of cells binding the antibody.
2. Skin rash and itching: A frequent complication which is probably due to minor allergic reactions to rabbit globulin. These symptoms will usually be prevented by or controlled with anti-histamines as well as with concomitant administration of corticosteroids.
3. Anaphylaxis: A rare but severe allergic reaction which may cause a life threatening drop in blood pressure, wheezing and difficulty breathing and severe hives. This complication can be treated with anti-histamines and steroids.
4. Platelet and white cell count depression: These are frequently observed and are probably caused by the binding of the rabbit antibody to human blood elements. Platelet transfusions will be administered to reduce the chance of bleeding or life threatening hemorrhage.
5. Serum sickness: Approximately 30% of patients treated with rabbit globulin will develop a late immune reaction to the globulin resulting in serum sickness 3-10 days after the administration. This may lead to severe skin rashes, mouth and vaginal sores, pain and swelling of the joints, or kidney damage. Serum sickness is transient and its damage reversible but it may require prolonged treatment with corticosteroids.

CHAPTER 3

3. STUDY ENDPOINTS

3.1 Definition of Morphologic Complete Remission

Complete remission (CR) will be defined as all of the following according to the revised recommendations of the international working group:[74]

- A bone marrow aspirate containing spicules with < 5% blasts with a count of at least 200 nucleated cells and no Auer rods seen. If spicules are absent in the aspirate, a bone marrow biopsy should confirm that < 5% blasts are present.
- No evidence of a persistently abnormal leukemic population by flow cytometry.
- ANC > 1000/μL and platelet count > 100,000/μL.
- No extramedullary leukemia.
- No blasts in peripheral blood.

3.2 Definition of Morphologic Relapse

Relapse will be defined as any of the following according to the revised recommendations of the international work group: [74]

- The reappearance of leukemia blast cells in the peripheral blood.
- > 5% blasts in the marrow, not attributable to another cause (e.g., bone marrow regeneration).
- The appearance of new dysplastic changes within the bone marrow.
- If there are no circulating blasts, but the marrow contains 5-20% blasts, a repeat bone marrow ≥ 1 week later with > 5% blasts is necessary to meet the criteria for relapse.
- The development of extramedullary leukemia or leukemic cells in the cerebral spinal fluid.

3.3 Primary Endpoint

The primary objective is to assess the probability of disease-free survival (DFS) at 6 months post-transplant. Death or relapse will be considered events for this endpoint.

3.4 Secondary Endpoints

3.4.1 Leukemia Relapse

Time to relapse will be calculated from the time of transplant to evidence of relapse as defined above.

3.4.2 Neutrophil Engraftment

Time to neutrophil engraftment is measured by determining the first of three consecutive measurements of ANC ≥ 500/μL following conditioning regimen induced nadir, starting from Day 0.

3.4.3 Platelet Engraftment

Time to platelet engraftment is measured by determining the first of three consecutive measurements of platelet count ≥ 20,000/μL without platelet transfusion support for seven days, starting from Day 0.

3.4.4 Graft Failure

Primary graft failure is defined as the failure to achieve an absolute ANC > 500 cells/μL by Day +30. Secondary graft failure is defined as initial neutrophil engraftment followed by subsequent decline in neutrophil counts < 500 cells/μL, unresponsive to growth factor therapy.

3.4.5 Acute Graft-versus-host Disease

Incidence and severity of acute GVHD will be graded according to the BMT CTN MOP.

3.4.6 Chronic Graft-versus-host Disease

Incidence and severity of chronic GVHD will be scored according to the BMT CTN MOP.

3.4.7 Transplant Related Mortality

Death occurring in a patient in continuing complete remission.

3.4.8 Determination of Infusional Toxicity

Maximum toxicity on Day 0 will be evaluated by measuring the patient's blood pressure, heart rate, respirations and temperature one hour prior to the allograft infusion and then approximately 15 minutes, 30 minutes, 2 hours, and 4 hours post infusion.

3.4.9 Disease-free Survival

DFS is defined as the minimum time interval of times to relapse/recurrence, to death or to the last follow-up, from the time of transplant.

3.4.10 Overall Survival

Overall survival is defined as time from transplant to death or last follow-up.

3.4.11 $CD34^+$ and $CD3^+$ Cell Doses

Total $CD34^+$ and $CD3^+$ cell doses will be calculated based on results of flow cytometric analysis.

3.4.12 Post-transplant Lymphoproliferative Disorder (PTLD)

PTLD is defined as increased EBV viremia requiring clinical intervention.

CHAPTER 4

4. PATIENT AND DONOR ENROLLMENT AND EVALUATION

4.1 Enrollment

Patients will be registered using AdvantageEDC^{SM}, an Internet data entry system. The following procedures should be followed:

1. An authorized user at the clinical center completes a screening form with demographic and primary eligibility questions. Eligibility includes questions confirming that the patient and donor have signed an informed consent form.
2. If the patient is eligible, a study number is generated.
3. A visit schedule based on treatment start date is displayed for printing.

If a connection is interrupted during a registration session, the process is completely canceled and logged. A backup manual registration system will also be available to provide for short-term system failure or unavailability.

4.2 Study Monitoring

4.2.1 Follow-up Schedule

The schedule for follow-up visits is outlined in Table 4.2.1.

TABLE 4.2.1: FOLLOW-UP SCHEDULE

Study Visit	Target Day Post-Transplant
1 week	7 ± 2 days
2 week	14 ± 2 days
3 week	21 ± 2 days
4 week	28 ± 2 days
5 week	35 ± 2 days
6 week	42 ± 2 days
7 week	49 ± 2 days
8 week	56 ± 2 days
9 week	63 ± 2 days
10 week	70 ± 2 days
11 week	77 ± 2 days
12 week	84 ± 2 days
13 week	91 ± 2 days
100 day	100± 2 days
6 month	180 ± 28 days
9 month	270 ± 28 days
12 month	365 ± 28 days
24 month	730 ± 28 days

Criteria for Forms Submission: Criteria for timeliness of submission for all study forms are detailed in the Data Management Handbook and User's Guide. Forms that are not entered into the web-based data entry system within the specified time will be considered delinquent. A missing form will continue to be requested either until the form is entered into the web based data entry system and integrated into the DCC's master database, or until an exception is granted and entered into the Missing Form Exception File, as detailed in the Data Management Handbook and User's Guide.

Reporting Patient Deaths: Recipient Death Information must be entered into the web-based data entry system within 1 business day of knowledge of the patient's death. If the cause of death is unknown at that time, it need not be recorded at that time. However, once the cause of death is determined, the form must be updated in the web based data entry system.

IBMTR Data Reporting: All transplant centers will be required to **register** all transplant recipients at their institutions with the IBMTR. Additionally, the IBMTR Day 100 Report Form (including the Core, Graft and Disease Inserts) and IBMTR Follow-up Form (including the Core and Disease Inserts) will be submitted directly to the IBMTR at the times specified in the Data Management Handbook and User's Guide for each patient enrolled on this study.

Adverse Event (AE) Reporting: Unexpected grade 3 - 5 adverse events will be reported through an expedited AE reporting system. Unexpected, grade 4 - 5 AEs must be reported

within 24 hours of knowledge of the event. Unexpected grade 3 AEs must be reported within three business days of knowledge of the event. Expected adverse events will be reported using NCI's Common Terminology Criteria for Adverse Events (CTCAE) Version 3.0 at regular intervals as defined on the Form Submission Schedule.

Weekly GVHD Monitoring: GVHD should be monitored in accordance with BMT CTN guidelines as specified in the Manual of Procedures. Patients should be assessed weekly until Day 100 post-transplant for GVHD. After Day 100, patients will be assessed at each study visit for the presence of GVHD.

4.2.2 Patient Assessments

Table 4.2.2 summarizes the patient clinical assessments over the course of the study.

4.2.2.1 Pre-transplant

The following observations are required for the study and must be **within 4 weeks prior** to the initiation of conditioning therapy. They are considered standard of care.

1. Physical examination, height and weight.
2. Infectious disease titers including cytomegalovirus (CMV) antibody test, hepatitis panel (Hepatitis B including HBsAg, HBcAb [IgM and IgG]; Hepatitis C ab), HIV including HIV Ag, HIV 1+2 Ab, HTLV I/II Ab, syphilis, Epstein-Barr Virus (EBV), herpes simplex virus (HSV), varicella zoster virus (VZV).
3. Cardiac performance (LVEF) determined by MUGA or echocardiogram.
4. DLCO.
5. Lumbar puncture if prior history of CNS disease.
6. Bone marrow biopsy/aspirate for confirmation of remission status. Original diagnostic bone marrow aspirate and/or biopsy should also be reviewed at each site to confirm the diagnosis of AML.
7. Serum or urine Beta HCG for female recipients.
8. Quantitative immunoglobulins for IgG, IgA, IgM.
9. HLA typing: At a minimum, serologic typing for HLA-A, -B and C loci and low resolution molecular testing for HLA-DRB1 (this may be done more than 4 weeks prior to conditioning).
10. Sample of peripheral blood to site laboratory for chimerism studies according to institutional standards. An initial pre-transplant sample is collected to identify informative genetic differences between patient and donor for subsequent analyses. The site laboratory may reference these studies to a designated appropriate source lab. Additional studies may be included per investigator's discretion for institutional follow-up practices.

The following observations are required for the study and must be **within 1 week prior** to the initiation of conditioning therapy. They are considered standard of care.

1. History.
2. Vital signs including blood pressure, pulse rate, respiratory rate and temperature.
3. Karnofsky performance status.
4. Automated CBC (WBC, RBC, hematocrit, hemoglobin) with differential and platelet count.
5. Serum chemistries panel including electrolytes, glucose, BUN, ALT, AST, creatinine, bilirubin, alkaline phosphatase, LDH, albumin. Electrolytes to include sodium, potassium, chloride, carbon dioxide, calcium and magnesium.

4.2.2.2 Pre-transplant and post-initiation of conditioning regimen

The following evaluations will occur during conditioning therapy (Day -10 to Day -1). They are considered standard of care.

1. Automated CBC (WBC, RBC, hematocrit, hemoglobin) with differential and platelet count daily while hospitalized is recommended.
2. Serum chemistries panel including electrolytes, glucose, BUN, ALT, AST, creatinine, bilirubin, alkaline phosphatase, LDH, albumin, on at least the first and last day of conditioning therapy. Electrolytes to include sodium, potassium, chloride, carbon dioxide, calcium and magnesium.

The following additional evaluations will occur on the day of $CD34^+$ cell infusion (Day 0). They are considered standard of care.

1. Vital signs including blood pressure, pulse rate, respiratory rate, temperature and pulse oximetry within 1 hour prior to starting infusion and at 15, 30, 120 and 240 minutes post-infusion.
2. Automated CBC (WBC, RBC, hematocrit, hemoglobin) with differential and platelet count on the day of infusion, but prior to infusion.
3. Serum chemistries panel including electrolytes, glucose, BUN, ALT, AST, creatinine, bilirubin, alkaline phosphatase, LDH, albumin. Electrolytes to include sodium, potassium, chloride, carbon dioxide, calcium and magnesium. These assessments are to be done on the day of infusion, but prior to infusion.

4.2.2.3 Post- transplant evaluations

The following evaluations will occur starting the day after transplant. They are considered both standard of care and research as specified below.

STANDARD OF CARE:

1. Physical examination, including Karnofsky performance status, on Day 28, 63, 100, 180 and 365.
2. Vital signs including blood pressure, pulse rate, respiratory rate and temperature on Day 28, 63, 100, 180 and 365.
3. Automated CBC (WBC, RBC, hematocrit, hemoglobin) with differential and platelet count on a daily basis during the post-infusion period while hospitalized is recommended. It is required once weekly after discharge until Day 28, then on Day 63, 100, 180 and 365, to obtain information to document engraftment.
4. Serum chemistries panel including glucose, electrolytes, BUN, ALT, AST, creatinine, bilirubin, alkaline phosphatase, LDH, albumin once weekly until Day 28, then on Day 63, 100, 180 and 365. Electrolytes to include sodium, potassium, chloride, carbon dioxide, calcium and magnesium.
5. Assessment of relapse of disease using bone marrow aspirate/biopsy or other tests as clinically indicated on other days but mandatory on Day 100, 180 and 365.
6. CMV surveillance performed weekly until Day 100 and then at least monthly through Day 180.
7. Quantitative immunoglobulin levels (performed at site) to include IgG, IgA, IgM and on Day 63, 100, 180 and 365.
8. Peripheral blood samples to site laboratory for T-lymphoid and myeloid lineage specific chimerism studies on Day 28, 100, 180 and 365. The site laboratory may reference these studies to an appropriate designated source lab. Additional studies may be included per investigator's discretion for institutional follow-up practices.
9. Complete acute GVHD staging and information including assessments of rash, diarrhea, nausea/vomiting, weight and liver function tests daily while hospitalized and once weekly after discharge until Day 100, then on Day 180 and 365.
10. Assessment for the presence of chronic GVHD at 3, 4, 6, 12, 24 months post-transplant.

RESEARCH ASSESSMENTS:

1. EBV surveillance by quantitative PCR performed weekly until Day 100 and then at least monthly through Day 180.
2. Peripheral blood samples for immunophenotyping at Day 28, 100, 180 and 365.

TABLE 4.2.2: PATIENT CLINICAL ASSESSMENTS OVER THE COURSE OF THE STUDY

Study Assessments/ Testing	Baseline[1]	Conditioning[2]	Day 0	Days Post-Transplant																	
				7	14	21	28	35	42	49	56	63	70	77	84	91	98	100	120	180	365	730
History, physical exam, height and weight[3]	X						X					X						X		X	X	
Vital signs[4]	X		X[5]				X					X						X		X	X	
Karnofsky performance status	X						X					X						X		X	X	
Automated CBC with differential, platelet count	X		X	X[6]	X[6]	X[6]	X[6]					X						X		X	X	
Serum chemistries panel[7]	X	X[8]	X	X	X	X	X					X						X		X	X	
Infectious disease titers[9]	X																					
LVEF[10]	X																					
DLCO	X																					
EBV and CMV Surveillance				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	At least monthly			
Bone marrow aspirate/biopsy[11]	X																	X		X	X	
ß-HCG serum pregnancy test (females only)	X																					
Quantitative Immunoglobulins for IgG, IgA and IgM	X											X						X		X	X	
Promthrombin time (PT), Partial Thromboplastin time (PTT)	X																					
5 mL heparinzed blood for HLA typing	X																					
Peripheral blood sample for chimerism	X						X[12]											X[12]		X[12]	X[12]	
Peripheral blood sample for immunophenotyping							X											X		X	X	
Pre- and post-selection sample of leukapheresis product for graft evaluation[13]		X																				
Acute GVHD and other morbidity assessments[14]				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X
Chronic GVHD																		X	X	X	X	X

Notes for Table 4.2.2 on next page.

Notes for Table 4.2.2:

[1] Baseline refers to the period prior to conditioning. Assessments should be made within 4 weeks prior to day of conditioning except for history, vital signs, Karnofsky performance, automated CBC with differential and platelet count, and serum chemistries panel which should be within 1 week prior to day of conditioning.

[2] Assessments should be made during the conditioning regimen period on Day -10 to Day -1.

[3] History and height are required only at baseline.

[4] Vital signs: blood pressure, pulse rate, respiratory rate and temperature

[5] Vital signs as listed above and including pulse oximetry within 1 hour prior to starting infusion and at 15, 30, 120 and 240 minutes post-infusion

[6] Daily during the post-infusion period while hospitalized (recommended) and once weekly after discharge until Day 28.

[7] Serum chemistries panel: electrolytes, glucose, BUN, ALT, AST, creatinine, bilirubin, alkaline phosphatase, LDH, albumin. Electrolytes to include sodium, potassium, chloride, carbon dioxide, calcium and magnesium.

[8] Required on the first and last days of the conditioning regimen.

[9] Infectious disease titers: Cytomegalovirus (CMV) antibody test, hepatitis panel (Hepatitis B including HBsAg, HBcAb (IgM and IgG); Hepatitis C ab), HIV including HIV Ag, HIV 1+2 Ab, HTLV I/II Ab, syphilis, Epstein-Barr Virus (EBV), herpes simplex virus (HSV), varicella zoster virus (VZV)

[10] To be determined by MUGA or echocardiogram.

[11] For assessment of relapse of disease (after baseline assessment). Mandatory on Days +100, +180 and +365. Other tests as clinically indicated on other days may be performed.

[12] Peripheral blood samples sent to site laboratory for T-lymphoid and myeloid lineage specific chimerism studies.

[13] Leukapheresis to begin on Day -1. Refer to Section 2.5.2.3 for required assessments for graft evaluation and criteria for release of product.

[14] Daily while hospitalized and weekly upon discharge.

4.2.3 Graft Evaluation

Samples will be taken from each leukapheresis product pre- and post- $CD34^+$ selection and characterized as per Section 2.5.2.3.

4.2.4 Donor Assessments

The following laboratory tests/evaluations will be performed for all donors registered in the study within 28 days prior to the day of transplantation. Additional evaluations/studies may also be performed by the site as dictated by the donor's clinical situation or according to each institution's standard practice for monitoring normal donors. Table 4.2.4 summarizes the donor clinical assessments.

4.2.4.1 Baseline

Baseline donor evaluation will be within 28 days of transplantation (Day 0). The evaluation will include:

1. History and physical examination.
2. Vital signs including blood pressure, pulse rate, respiratory rate and temperature.
3. Automated CBC (WBC, RBC, hematocrit, hemoglobin) with differential and platelet counts.
4. Serum chemistries panel including electrolytes, glucose, BUN, ALT, creatinine, bilirubin, alkaline phosphatase, LDH and albumin. Electrolytes to include sodium, potassium, chloride, carbon dioxide, calcium and magnesium.
5. Infections disease titers including CMV antibody test, hepatitis panel (Hepatitis B including HBsAg, HBcAb [IgM and IgG]; Hepatitis C ab), NAT testing for HIV 1 and Hepatitis C, HIV 1+2 Ab, HTLV I/II Ab, syphilis, EBV Ab HSV Ab, VZV Ab.
6. Serum or urine Beta HCG for female donors.
7. Prothrombin time (PT) and partial thromboplastin time (PTT).
8. Urinalysis is recommended.
9. Serologies for HLA Typing: HLA-A, B, C loci (typed at least at the serologic level) and HLA-DRB1 at least at low resolution molecular level using DNA typing techniques (by PCR/SSOP if available).
10. Baseline samples for chimerism studies to the site laboratory. DNA studies for sex-matched donor/recipient pairs. For sex mismatched donor/recipient pairs, cytogenetic studies or FISH are acceptable.

4.2.4.2 During mobilization phase

Automated CBC with differential is required on the first and last day of mobilization therapy.

4.2.4.3 During apheresis and cell processing

Donor lab assessments should be in accordance with institutional standard operating procedures, but should include at a minimum the following:

1. Automated CBC with differential pre- and immediately post-leukapheresis on each day of leukapheresis.
2. Electrolytes to include sodium, potassium, chloride, carbon dioxide, calcium and magnesium immediately prior to leukapheresis on each day of leukapheresis.
3. Toxicity evaluation performed on the last day of G-CSF administration.

4.2.4.4 Post apheresis

Donors must be contacted by phone approximately 30 days post initiation of G-CSF for a toxicity evaluation.

TABLE 4.2.4: SUMMARY OF DONOR CLINICAL ASSESSMENTS

ASSESSMENTS	Baseline[1]	Mobilization Phase	Apheresis
History and physical exam	X		
Vital signs including blood pressure, pulse rate, respiratory rate and temperature	X		
Automated CBC with differential, platelet count	X	X[2]	
Serum chemistries panel[3]	X	X[3a]	
Infectious disease titers[4]	X		
ß -HCG serum pregnancy test (females only)	X		
Promthrombin time (PT), Partial Thromboplastin time (PTT)	X		
Urinalysis (recommended)	X		
HLA typing[5]	X		
Baseline sample for chimerism[6]	X		
Toxicity evaluation			X[7]

[1] Baseline refers to the period prior to conditioning. Assessments should be made within 4 weeks prior to start of transplantation.
[2] Only automated CBC with differential is required on the first and last days of mobilization therapy and each day of leukapheresis.
[3] Serum Chemistries Panel: electrolytes, glucose, BUN, ALT, creatinine, bilirubin, alkaline phosphatase, LDH, albumin. Electrolytes to include sodium, potassium, chloride, carbon dioxide, calcium and magnesium.
[3a] Electrolytes to include sodium, potassium, chloride, carbon dioxide, calcium and magnesium immediately prior to leukapheresis on each day of leukapheresis.
[4] Infectious Disease Titers: CMV antibody test, hepatitis panel (Hepatitis B including HBsAg, HBcAb (IgM and IgG); Hepatitis C Ab), HIV including HIV Ag, HIV 1+2 Ab, HTLV I/II Ab, syphilis, EBV Ab, HSV Ab, VZV Ab
[5] HLA-A, B and C loci typed at least at the serologic level and HLA-DRB1 at low resolution molecular level using DNA typing techniques.
[6] DNA studies for sex-matched donor/recipient pairs. For sex mis-matched donor/recipient pairs cytogenetic studies or FISH are acceptable.
[7] On Days 5 and 30 (via telephone) post initiation of G-CSF.

CHAPTER 5

5. STATISTICAL CONSIDERATIONS

5.1 Study Design

The study is a Phase II, single arm, multicenter trial. It is designed to assess whether the anticipated endpoints for a T cell depleted transplant arm of a planned prospective randomized trial comparing T cell depleted and non-TCD allografts are likely to be achieved in a multicenter study conducted by the BMT CTN. The study population is patients with AML in CR1 or CR2. The sample size is 45 patients for this trial.

5.2 Accrual

It is estimated that one year of accrual will be necessary to enroll the targeted sample size. Accrual will be reported by race, ethnicity, gender, and age.

5.3 Study Duration

Patients will be followed for a minimum of two years post-transplant. Patients who do not receive a transplant or patients whose grafts are either not appropriately T cell depleted ($CD3^+$ cell dose > 1.0×10^5/kg) or contain < 1.0×10^6/kg $CD34^+$ cells despite up to three donor leukapheresis procedures, will be considered under the intent to treat principle and will be evaluated for the primary endpoint. A secondary analysis will also be performed including only patients with evaluable grafts ($CD34^+$ cell dose > 1.0×10^6/kg and $CD3^+$ cell dose < 1.0×10^5/kg).

Patients who experience relapse will be considered as reaching the primary study endpoint. These patients must continue to be followed for survival.

5.4 Randomization

There is no randomization aspect to this trial.

5.5 Primary Objective

The primary objective is to assess the disease-free survival (DFS) probability at 6 months post-transplant. Death or relapse will be considered events for this endpoint.

5.6 Sample Size and Power Considerations

The sample size is 45 patients for this trial. Ninety-five percent confidence intervals were calculated for varying probabilities based on the sample size. Table 5.6.1 provides confidence intervals for a variety of true underlying proportions. Of particular interest is where the DFS probability is 75%, which is the anticipated 6-month DFS probability. For this setting, the confidence interval length is 25.3%. The percentages above and below 75% are meant to represent other plausible DFS percentages.

The precision of the estimates alternatively could be viewed as a lower bound on the rate of DFS. The probability to rule out DFS percentages of a certain size is known as "power". Table 5.6.2 provides the probability (or power) that the lower bound of a 95% two-sided confidence interval for the DFS probability will be greater than a threshold of 90%, 80%, 70%, or 60%. When the true percentage is 75%, there is 84% power to rule out a DFS percentage of < 55%.

The lower bound for proceeding to a Phase III trial is DFS probability > 0.55. This can also be viewed as testing the following hypothesis: H_o: $p \leq 0.55$ versus H_1: $p > 0.55$. Based on the table below, there is 84% power at $\alpha = .05$ to reject the null if the true percentage is 75%.

TABLE 5.6.1: CONFIDENCE INTERVAL LENGTHS AND POSSIBLE CONFIDENCE INTERVALS FOR VARIOUS UNDERLYING DISEASE-FREE SURVIVAL PROBABILITIES

N	Disease-free Survival %	Length of 95% Confidence Interval	Possible Confidence Intervals	
45	90	17.5	81.2	98.8
45	80	23.4	68.3	91.7
45	75	25.3	62.3	87.7
45	70	26.8	56.6	83.3
45	60	28.6	45.7	74.3

The DFS probability estimate will be based on the Kaplan-Meier product limit estimator using Greenwood's formula as the variance estimate. In the absence of censoring, the Kaplan-Meier estimate reduces to the simple binomial proportion.

TABLE 5.6.2: PROBABILITY OF RULING OUT A THRESHOLD OF SIZE T OR LARGER FOR VARIOUS TRUE UNDERLYING DISEASE-FREE SURVIVAL PERCENTAGES

N	True Disease-Free Survival %	Probability of Ruling Out Disease-Free Survival Percentages of Size T or Smaller				
		T=90%	T=80%	T=75%	T=65%	T=55%
45	90		0.58	0.83	0.99	1.00
45	80	0.35		0.15	0.68	0.96
45	75	0.70	0.10		0.35	0.84
45	65	0.98	0.57	0.27		0.30
45	55		0.96	0.80	0.26	

5.7 Interim Analysis and Stopping Guidelines

Interim analyses for efficacy will be conducted at times coincident with regularly scheduled meetings of the NHLBI-appointed Data and Safety Monitoring Board (DSMB) at approximately six-month intervals. Monitoring of key safety endpoints (transplant-related mortality [TRM], graft failure, GVHD) will be conducted monthly, and if rates significantly exceed pre-set thresholds, the NHLBI will be notified in order that the DSMB can be advised. Policies and composition of the DSMB are described in the BMT CTN's Manual of Procedures. The stopping guidelines serve as a trigger for consultation with the DSMB for additional review, and are not formal "stopping rules" that would mandate automatic closure of study enrollment.

The rate of TRM will be monitored up to 100 days post-transplant. Monitoring will be performed monthly beginning after the third month of enrollment until enrollment is closed. At least three events must be observed in order to trigger review. Each month, the null hypothesis that the 100-day TRM rate is less than or equal to 10% is tested. An extension of the sequential probability ratio test (SPRT) will be used to monitor TRM. A description of the SPRT is provided below.

The SPRT can be represented graphically. At each monthly interim analysis, the total time on study is plotted against the total number of patients experiencing TRM. The continuation region of the SPRT is defined by two parallel lines. Only the lower boundary will be used for monitoring to protect against excessive 100-day TRM. If the graph falls below the lower boundary, the SPRT rejects the null hypothesis, and concludes that there are more events than predicted by the observed time on study. Otherwise, the SPRT continues until enrollment reaches the maximum of 45 patients.

This procedure assumes an exponential distribution for the time until TRM during the first 100 days, but censors follow-up time after 100 days. Only events that occur on or before the patient has been followed for 100 days are counted. Total time on study is computed as time from registration to event, or to 100 days, whichever comes first, summed for all evaluable patients on study.

The usual measures of performance of an SPRT are the error probabilities α and β of rejecting H_0 when $\theta = \theta_0$ and of accepting H_1 when $\theta = \theta_1$, respectively, and the expected sample size $E(N|\theta_i)$. The test to be used in this protocol was developed from the following SPRTs: A SPRT contrasting 10% versus 30% 100-day rate of TRM results in a common slope of 1.33 and the intercepts are –2.27 and 1.64.

The actual operating characteristics of the truncated test, shown in Table 5.7.1, were determined in a simulation study that assumed uniform accrual of 45 individuals over a one-year time period, and exponential time to failure after registration. Since 100,000 replications were used, the estimates have two digits of precision.

TABLE 5.7.1: OPERATING CHARACTERISTICS OF SEQUENTIAL TESTING PROCEDURE FROM A SIMULATION STUDY WITH 100,000 REPLICATIONS FOR TRANSPLANT RELATED MORTALITY (TRM)

True 100-Day Rate	10%	20%	30%
Probability Reject Null	0.05	0.50	0.92
Mean Month Stopped	12.7	10.08	6.74
Mean # Endpoints in 100 Days	4.00	6.00	6.00
Mean # Patients Enrolled	44	36	25

The testing procedure for TRM rejects the null hypothesis in favor of the alternative 5% of the time when the true 100-day TRM rate is 10%, and 92% of the time when the rate is 30%. This corresponds to a type I error rate of $\alpha = 0.05$ and a type II error rate of $\beta = 0.08$. When the true 100-day TRM rate is 30%, on average, the DSMB will be consulted 6.74 months after opening, when 6 events have been observed in 25 patients.

Acute GVHD grades III-IV will also be monitored, testing the null hypothesis of 100-day post-transplant rate of acute GVHD less than or equal to 5%. The following are the operating characteristics for acute GVHD grades III-IV if the stopping guideline is observing the five events in Table 5.7.2.

TABLE 5.7.2: OPERATING CHARACTERISTICS FROM A SIMULATION STUDY WITH 100,000 REPLICATIONS FOR ACUTE GVHD GRADES III-IV IF STOP AT FIVE EVENTS

True 100-Day Rate	5%	10%	15%	20%
Probability Reject Null	0.05	0.35	0.72	0.91
Mean Month Stopped	11.92	11.19	9.82	8.39
Mean # Endpoints in 100 Days	1.93	3.62	4.69	5.20
Mean # Patients Enrolled	45	42	37	32

The testing procedure for acute GVHD grades III-IV rejects the null hypothesis in favor of the alternative 5% of the time when the true 100-day acute GVHD grade III-IV rate is 5% and 91% of the time when the true rate is 20%. This corresponds to a type I error rate of $\alpha = 0.05$ and a type II error rate of $\beta = 0.09$. When the true 100-day acute GVHD grades III-IV rate is 20%, on average, the DSMB will be consulted 8.39 months after opening when 5.20 occurrences of acute GVHD grades III-IV have occurred in 32 patients.

Graft failure by Day 28 will be followed by testing the null hypothesis that the Day 28 post-transplant rate of graft failures is less than or equal to 1%. The following are the operating characteristics for graft failure if the stopping guideline is observing two events:

TABLE 5.7.3: OPERATING CHARACTERISTICS FROM A SIMULATION STUDY WITH 100,000 REPLICATIONS FOR GRAFT FAILURE IF STOP AT TWO EVENTS

True 28-Day Rate	1%	5%	11%
Probability Reject Null	0.08	0.66	0.97
Mean Month Stopped	12.68	9.23	5.60
Mean # Endpoints in 28 Days	0.44	1.63	2.16
Mean # Patients Enrolled	44	34	21

The testing procedure for graft failure rejects the null hypothesis in favor of the alternative 8% of the time when the true 28-day graft failure rate is 1% and 97% of the time when the rate is 11%. This corresponds to a type I error rate of $\alpha = 0.08$ and a type II error rate of $\beta = 0.03$. When the true 28-day graft failure rate is 11%, on average, the DSMB will be consulted 5.6 months after opening, when 2.16 graft failures have been observed in 21 patients.

5.8 Demographic and Baseline Characteristics

Demographics and baseline characteristics will be summarized for all patients. Characteristics to be examined are: age, gender, race/ethnicity, performance status, HLA match, disease stage, serum bilirubin level, serum creatinine level, donor age, donor gender, and donor ethnicity.

5.9 Analysis of Primary Endpoint

The primary analysis will consist of estimating the 6-month DFS (from day of enrollment) probability based on the Kaplan-Meier product limit estimator. The 6-month DFS probability and confidence interval will be calculated. All registered patients will be considered for this analysis. A secondary analysis will be performed including only patients receiving grafts with $CD34^+$ dose $> 1.0 \times 10^6$/kg and $CD3^+$ dose $< 1.0 \times 10^5$/kg.

5.10 Analysis of Secondary Endpoints

- **Time to Acute Leukemia Relapse:** To assess the incidence of acute leukemia relapse from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval. Death prior to relapse will be considered as a competing risk.

- **Time to Neutrophil Engraftment:** To assess the incidence of neutrophil engraftment from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval. Death prior to neutrophil engraftment will be considered as a competing risk.

- **Time to Platelet Engraftment:** To assess the incidence of platelet engraftment from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval. Death prior to platelet engraftment will be considered as a competing risk.

- **Time to Acute GVHD:** To assess the incidence and severity of grades II-IV acute GVHD from day of transplant. The first day of acute GVHD onset at a certain grade will be used to calculate a cumulative incidence curve for that acute GVHD grade. An overall cumulative incidence curve will be computed along with a 95% confidence interval at 100 days post-transplant with death considered as a competing risk.

- **Time to First Clinical Onset of Chronic GVHD:** To assess the incidence and severity of chronic GVHD from day of transplant, a cumulative incidence curve will be computed along with a 95% confidence interval at two years post-transplant. Death prior to occurrence of chronic GVHD will be considered as a competing risk.

- **Transplant Related Mortality (TRM):** TRM is death occurring in patients in continuous complete remission. The TRM distribution will be estimated by the Kaplan-Meier curve at two years post-transplant.

- **Overall Survival:** The survival distribution will be estimated by the Kaplan-Meier curve. All patients will be followed for a minimum of two years post-transplant for mortality.

- **$CD34^+$ and $CD3^+$ Cell Doses:** The proportions (and 95% confidence intervals) of patients receiving grafts with specified $CD34^+$ and $CD3^+$ cell doses will be calculated. The three proportions of interest are:

  1. Proportion receiving $CD34^+$ cell dose > 5×10^6/kg and $CD3^+$ cell dose < 1×10^5/kg
  2. Proportion receiving $CD34^+$ cell dose < 2×10^6/kg
  3. Proportion receiving $CD3^+$ cell dose > 1×10^5/kg

- **PTLD:** The proportion (and 95% confidence intervals) of patients who develop PTLD will be calculated.

5.11 Secondary Analysis

Transplant outcomes will be compared between patients in first complete remission as compared to patients in second complete remission. Outcomes will also be compared between patients who must receive a third unmanipulated product to the other patients who do not receive an unmanipulated product. The power for these two comparisons will be very small. However, these comparisons were requested by the FDA.

5.12 Safety Analysis

The reporting of serious adverse events will be consistent with standard BMT CTN procedures. The type and severity of adverse events will be analyzed.

APPENDIX A

CLASSIFICATION OF ACUTE MYELOID LEUKEMIA

APPENDIX A

Classification of Acute Myeloid Leukemia

Patients should be classified by either the FAB (French-American-British Classification) or the World Health Organization (WHO) classification as indicated below:

I. FAB Classification

M0: Acute myeloid leukemia with minimal differentiation

M1: Acute myeloid leukemia without maturation

M2: Acute myeloid leukemia with maturation

M3: Acute promyelocytic leukemia

M4: Acute myelomonocytic leukemia

M4E: Acute myelomonocytic leukemia with abnormal eosinophils

M5A: Acute monoblastic leukemia (poorly differentiated)

M5B: Acute monocytic leukemia (differentiated)

M6: Acute erythroleukemia

M7: Acute megakaryoblastic leukemia

RAEBT: Refractory anemia with excess blasts in transformation. Patients with this FAB classification subtype are eligible for this protocol, as long as they had > 20% blasts in the blood and/or marrow at initial diagnosis.

II. WHO Classification

A. AML WITH RECURRENT CYTOGENETIC TRANSLOCATION

Acute myeloid leukemia with t(8;21)(q22;q22), (*AML1/ETO*)

Acute myeloid leukemia with abnormal bone marrow eosinophils and inv(16)(p13q22) or t(16;16)(p13;q22), (*CBF_/MYH11*)

Acute promyelocytic leukemia with t(15;17)(q22;q12), (*PML/RAR_*) and variants

Acute myeloid leukemia with 11q23 (*MLL*) abnormalities

B. AML WITH MULTILINEAGE DYSPLASIA

Following MDS or MDS/MPD

Without antecedent MDS or MDS/MPD, but with dysplasia in at least 50% of cells in 2 or more myeloid lineages

C. AML, THERAPY RELATED

Alkylating agent/radiation–related type

Topoisomerase II inhibitor–related type (some may be lymphoid)

Others

D. AML, NOT OTHERWISE CATEGORIZED

Acute myeloid leukemia, minimally differentiated

Acute myeloid leukemia without maturation

Acute myeloid leukemia with maturation

Acute myelomonocytic leukemia

Acute monoblastic/acute monocytic leukemia

Acute erythroid leukemia (erythroid/myeloid and pure erythroleukemia)

Acute megakaryoblastic leukemia

Acute basophilic leukemia

Acute panmyelosis with myelofibrosis

APPENDIX B-1

RECIPIENT INFORMED CONSENT

Informed Consent to Participate in Research

We invite you to participate in this research study. About 45 patients will participate at up to eight centers around the country. Your study participation will last about two years. This is a study for patients who are going to have an allogeneic (donor) peripheral blood stem cell transplant for acute myelogenous leukemia.

This consent form tells you about the study. The doctors in charge of this study (the investigators) or other staff will also discuss this study with you and answer any questions you might have. Before you decide to join this study, please read this information and ask any questions about things you do not understand. Some patients find it helpful to have a family member or friend with them to help ask questions and listen to information.

This study will give more information to doctors about future treatment choices for patients with leukemia. It is important to know that:

- You will not be paid to be in this study.
- You or your insurance company will pay for all medical bills for your treatment.
- You will not be charged for research tests – tests you would not normally have if you were not a part of this study.

Before you decide to join the study, please read the information below. Feel free to ask questions to understand your rights. It is your choice to take part in this study. You and your doctor will discuss other treatment options if you decide not to be in this study.

Your Name: _____

1. Title of Research Study

A Single Arm, Multicenter Phase II Trial of Transplants of HLA-Matched, $CD34^+$ Enriched, T cell Depleted Peripheral Blood Stem Cells Isolated by the CliniMACS System in the Treatment of Patients with AML in First or Second Morphologic Complete Remission

2. Principal Investigator Contact Information at your Institution

Name/Title/Phone number/

3. Contact Information for Emergencies After Hours or on Weekends or Holidays
Name/Phone number/

4. Study Sponsors
This study is sponsored by the National Institutes of Health (NIH) by providing financial support for this study through the Blood and Marrow Transplant Clinical Trials Network (BMT CTN).

The device that removes T cells from the donor peripheral blood stem cells that is being used in this study is called the CliniMACS device. It is being supplied by Miltenyi Inc., the company that makes it. This company did not plan or design the study. In addition, they will have no part in analyzing the results of this study.

5. What is the purpose of this study?
A donor peripheral blood stem cell transplant has been offered to you because your doctors think it may improve the chances of curing your acute leukemia. Based on what we now know, when transplantation using a related donor is performed for patients with acute leukemia in remission, the chance of the leukemia returning is still present but less likely than with anti-cancer drugs alone. Anti-cancer drugs are usually called chemotherapy. Your doctor can discuss with you the results of other studies using chemotherapy alone without transplantation.

One of the possible complications of allogeneic stem cell transplant is graft-versus-host disease (GVHD). GVHD develops when T cells in the donor's peripheral blood stem cells (also called the graft), attack organs in your body, such as the skin, gastrointestinal tract (stomach and bowels), and liver. GVHD can also increase your risk of infection.

Signs of GVHD can include mild to severe skin rashes, yellowing of the skin (jaundice) due to liver disease, nausea and vomiting, mild to severe diarrhea and malnutrition and breathing problems. GVHD can be treated and often can be cured. Sometimes it continues despite treatment, but in a milder form. Although most of the time GVHD is treatable, sometimes patients can die because of complications due to GVHD.

Something must always be done to prevent GVHD, unless your donor is an identical twin. The most common approach to preventing GVHD is to give drugs to the patient receiving a transplant. This decreases the chance of getting GVHD and the severity of GVHD if it occurs. But it does not prevent GVHD in all patients. Approximately half of patients receiving donor transplants from a brother or sister still get GVHD.

Another method has been developed to remove T cells, the cells that cause GVHD, to prevent GVHD in more patients. If this is done, the patient does not have to receive drugs to prevent GVHD. T cells are removed in the laboratory, *after* the cells are collected from your donor, but *before* they are given to you. Several studies show that taking T cells out of the graft is good at preventing both the early and late forms of GVHD. This type of donor transplant is called a *T cell depleted transplant.*

It is possible that reducing your chances of getting GVHD by using T cell depletion may not improve your outcome after the transplant. Some methods of removing T cells from the donor cells have resulted in other bad outcomes. These outcomes include more patients rejecting the transplanted cells (graft failure), more patients' leukemia coming back after the transplant (relapse), an increase in potentially life threatening infections and possibly the development of another tumor. Previous studies in small numbers of patients using the device being tested in this study have not shown increased problems with graft rejection, leukemia relapse or new tumors. Your doctor can discuss these issues with you in more detail.

6. How will my treatment differ if I participate in this study?

The most likely treatment you would receive if you do not participate in this study would be a donor transplant from your brother or sister that has not had the T cells removed. The answer to this question lists what would be different for you if you participate in the study compared to that treatment.

a. You will receive a specific combination of medicines and irradiation to suppress your immune system prior to receiving your donor's cells. Although this combination has been used for this type of transplant for a long time, it is likely different from what you would receive if you do not participate in the study.

b. Most of the T cells will be removed from your donor's cells (the graft) before the graft is given to you. We will use the CliniMACS device to remove the T cells. This device uses a magnetic system to separate the stem cells away from the T cells that can cause GVHD.

c. You will not receive drugs after the transplant to prevent GVHD because GVHD should be prevented by removing the T cells from the donor graft using the CliniMACS device.

d. You will need to follow up with the doctors that do your transplant on a specific schedule for up to two years after your transplant.

7. What will be done if you take part in this research study?

The transplant process has many steps. You have been offered a T cell depleted peripheral blood stem cell transplant because you have a brother or sister that matches you and has agreed to be your donor. Both you and your donor will need to have tests done before you can have the transplant. You and your donor will need to give permission to join this study. Your donor may refuse to participate in this study, but continue to be available to donate cells for your transplantation**.** You may decide to have a transplant using this donor, but not join this study.

To make the consent form easier to follow, side effects and risks related to the different parts of the study treatments are given later in the consent form, after the description of the overall process.

Your participation in this study is expected to last up to two years. Initially, you will undergo a series of tests that include drawing about 6 tablespoons of blood. These tests are standard and are done to be sure it is safe for you to have the transplant. Samples of your bone marrow will be taken to be sure that your acute leukemia is in first or second remission. Samples of the fluid that bathes everyone's brain and spinal cord [cerebrospinal fluid] may also be

checked before transplant in special situations. This fluid is obtained by inserting a small needle in the lower middle part of your back; or if you have had a device placed in your head (Ommaya reservoir) for this purpose, fluid may be obtained from this site. It is necessary to check this fluid if you have a history of leukemia in your spinal fluid. It is also necessary if you have had leukemia anywhere outside your bone marrow such as in your skin or gums. These tests are always done for patients with acute leukemia who will receive a transplant. They are not being done just for this study's purposes.

You will then be admitted to the hospital and will have a catheter placed in a large blood vessel in the chest. This catheter placement will be done under local anesthesia and is considered standard of care for transplant patients. This catheter will be used to draw blood and give transfusions and medications. Your hospitalization and period of recovery following transplant can be broken down into four major parts.

The first part is the treatment that you are given in order to kill any leukemia cells in your body. This part also allows your body to accept your donor's peripheral blood stem cells. All patients receiving transplant must receive some kind of treatment to do this. However, the treatment you will receive is considered research since it is not routinely given to patients having a conventional peripheral blood stem cell transplant. However, it is felt that all the radiation and drugs given in the treatment are needed so that you will not reject your donor's peripheral blood stem cells. The first four days of treatment you will be given radiation treatment to your whole body, three times a day. After this, you will receive four days of chemotherapy. On the first two days, you receive a drug called thiotepa. The next two days, you receive a drug called cyclophosphamide. Both of these drugs will be given through your catheter. On the sixth day of treatment you will also receive a medicine called antithymocyte globulin or Thymoglobulin through your catheter. After the treatments are given, you will have a day off treatment before you receive your donor's peripheral blood stem cells.

The second part of your hospitalization is the peripheral blood stem cell transplant itself. The transplant is necessary to restore blood production after the high-doses of chemotherapy and radiation. Prior to your transplant, blood stem cells will be collected from your donor. The cells that can cause GVHD, T cells, will be removed from your donor's blood in the laboratory using a machine called the CliniMACS device. This is called T cell depletion. This part of the study is considered research because it is not routine to have T cells removed from your donor's blood stem cells. After the T cells are removed, the rest of the donor's blood stem cells are given to you in the same way you receive a blood transfusion. They are slowly injected into your veins through your catheter. It is not put directly back into your bone. The cells will find their own way to your bone marrow where they begin to grow again. Your donor's blood stem cells may have to be given to you on more than one day, after each collection.

The third major part of your hospitalization is the time during which the donated blood stem cells begin to grow and provide normal blood cells for you. During this time, patients develop many of the problems that occur because of the treatment they received before the transplant [see Risks, below]. These problems are, for the most part, related to the period in which neither you nor your donated blood stem cells are making enough normal blood cells for you.

These problems occur with all transplants like this and are not specifically related to this study. You may also have severe irritation on the insides of your mouth, esophagus or swallowing tube, stomach, and intestines. Until your immune system recovers and is able to fight infection on its own, steps will be take to protect you from being exposed to too many germs. Until your blood counts reach safe levels, you will be given blood & platelet transfusions. Once your donated blood stem cells begin to grow enough normal cells, much of this treatment can be stopped, and most patients can go home.

The fourth major part of your treatment is your recovery period. Many patients have no further problems and remain at home. Patients typically see their transplant doctor in the clinic on a regular, usually weekly, basis until at least about 100 days after the transplant and less frequently after that. Other patients may develop problems that require re-admission to the hospital (see Risks, below), like a fever or pneumonia or the development of GVHD. You might think of yourself as a newborn baby with little or no resistance or immunity to many common, everyday infections. For this reason, you will need to take all the preventative medicines prescribed for you when you leave the hospital. You will also need to take all precautions explained to you to prevent or reduce your chances of getting a fever or infection. Above all, you will need to stay in close contact with your transplant doctor.

You may require transfusions of blood and/or platelets as an outpatient for several weeks (or rarely for months) after transplant. You will have bone marrow biopsies done at approximately 3, 6, and 12 months after the transplant to determine if you have adequately accepted the donor's blood forming cells and to monitor the status of your acute leukemia. These tests are important for your medical care and are not considered research.

The study coordinators at your center will collect information from your medical chart about you and your health over two years. They will collect information every week for 100 days, then at 3 months, 6 months, 1 year, and 2 years.

8. Will you provide blood samples for research?

You will provide samples for research to determine how your immune system is recovering after the transplant. This will involve drawing about one teaspoon of blood at about 1, 3, 6, and 12 months after the transplant.

You will also provide about one teaspoon of blood weekly up to 100 days and monthly to 6 months. These samples will be used to see if you have any evidence of a virus called Epstein-Barr virus or EBV. EBV is a virus that can cause a cancer of the immune cells. This cancer is called lymphoproliferative disease or LPD. This happens in less than 5% of patients. If untreated this complication can be serious or even cause death. It can be treated and reversed with a medicine called rituximab. This complication is more common after a T cell depleted transplant than a non-T cell depleted transplant. Screening for EBV is often done for patients getting a T cell depleted transplant. For this study the tests will be performed at a single laboratory and are considered part of the research.

Neither you nor your insurance company will be billed for these research tests.

9. What are the possible discomforts and risks?

Likely Side Effects	Less Likely Side Effects	Rare Side Effects
What it means: This type of side effect may occur in 10% or more of patients. This means that 10 or more patients out of 100 get this.	**What it means:** This type of side effect may occur in 3-9% of patients. This means that 3 to 9 patients out of 100 might get this.	**What it means:** This type of side effect does not occur very often, but can occur in less that 2% of patients. This means that 1 or 2 patients out of 100 might get this.

Central venous catheter (Central line):

Less Likely Side Effects	Rare Side Effects
• Clotting of blood (treated with a medicine that dissolves clots) • Bleeding around the catheter • Infection in the tissues around the catheter or in the bloodstream • Skin redness at the catheter exit site, which may require treatment with an antibiotic	• A small chance of a puncture to the lung during placement of the catheter • A blood clot can form on the tip of the catheter, break off, and go into the lungs (pulmonary embolus), which could cause shortness of breath and pain

In general, patients do well with their catheters, but there are side effects. Even though local anesthetic is used while placing the central venous catheter in your body, this will cause some discomfort.

Blood samples are drawn frequently to follow the treatment and course of events. The risk is limited to the discomfort at the site of the needle insertion, although most of the time, blood will be drawn through the central venous catheter, which is generally painless.

Cyclophosphamide (Cytoxan):

Likely Side Effects	Less Likely Side Effects	Rare Side Effects
• Lower white blood cell count with increased risk of infection • Diarrhea (loose stools) • Vomiting (throwing up) • Liver damage • Lower sperm production in men • Hair loss • Nausea (feeling sick to your stomach) • Loss of appetite • Missing or stopping menstrual cycle in women • Infertility	• Bleeding from the bladder • Sores in mouth or on lips • Blood in urine • Decreased energy / tiredness, fatigue • Lower platelet count (mild) with increased risk of bleeding • Darkening of nail beds • Fetal damage if pregnancy occurs while taking Cyclophosphamide	• Lung fibrosis with cough and shortness of breath • Heart failure with high doses • Decrease in sodium level in the blood with high doses • Secondary cancers

Cyclophosphamide can cause bleeding in your bladder. Getting more fluid through a vein or your catheter and drinking extra liquids may prevent this. A drug called Mesna is given to prevent damage to the bladder, and the bladder may be irrigated (washed out) with a salt-water solution.

It is not know whether the use of Cyclophosphamide will cause additional side effects or problems with patient health in the future.

Total body Irradiation (TBI):

Likely Side Effects	Less Likely Side Effects	Rare Side Effects
• Diarrhea (loose stools) • Nausea (sick to the stomach) • Stomach cramps • Vomiting (throwing up) • Painful swelling of the parotid gland (salivary glands under the ears) for a few days • Short-term hair loss • Anemia • Infection • Bleeding • Cataracts • Sterility (inability to have children • Growth failure • Endocrinopathies (such as thyroid disease or diabetes) • Mouth sores	• Lung inflammation Pneumonia • Redness of the skin • Liver problems	• Risk of developing other cancers in the future as a consequence of having received the total body irradiation • Difficulty swallowing • Back problems • Kidney problems

Although TBI can theoretically cause abnormalities in children born to transplant survivors, the incidence of genetic abnormalities has not been reported to be greater than the general population. However, this is a potential risk and birth control should be used for a least one year after transplant to minimize risks of conceiving.

Thiotepa:

Likely Side Effects	Less Likely Side Effects	Rare Side Effects
• Lower white blood cell count with increased risk of infection • Diarrhea (loose stools) • Vomiting (throwing up) • Liver damage • Lower sperm production in men • Hair loss • Nausea (feeling sick to your stomach) • Loss of appetite • Missing or stopping menstrual cycle in women • Mouth/throat sores • Sterility (inability to have children)	• Liver abnormalities • Skin rash • Change in skin coloring • Risk of bleeding due to low platelet count	• Confusion • Disorientation

Antithymocyte Globulin (ATG): This is a preparation of antibodies that were produced by rabbits that were immunized with thymocytes (T cells) from human donors. As with any protein which comes from a different species (animal), injection of ATG may cause fever reactions. In earlier studies, some patients who developed GVHD and were given ATG at doses higher than doses given in this study developed an unusual lymphoma associated with a virus called EBV, and some patients died with this complication. The development of lymphomas has not been a problem in patients given lower doses of ATG before transplantation. Blood tests will be done in this study to monitor for the development of EBV infection. If the amount of EBV in the bloodstream is found to be above a certain level, a medication will be given to prevent the development of lymphoma.

Likely Side Effects	Less Likely Side Effects	Rare Side Effects
• Fever and chills • Lower platelet count that increases your risk of bleeding • Skin rashes	• Allergic reactions may include shortness of breath, fast heart rate, low blood pressure, and/or serum sickness (itching rash, facial swelling, lymph node swelling, joint pain, diarrhea, and nerve pain)	• There is also a rare risk of anaphylaxis, which is a severe and sometimes dangerous reaction to this drug. This may cause fainting, itching, skin rash or problems breathing. It could result in death. • There may also be other side effects that we do not know about

Bone Marrow Suppression: The main side effect of radiation treatment, thiotepa, and cyclophosphamide is the destruction of your own bone marrow. This is good to the extent that it kills the leukemia cells. But it also destroys the normal bone marrow that makes all the normal blood cells. This leads to increased chances of infection, bleeding, weakness, dizziness, difficulty breathing, and headaches and/or difficulty thinking clearly. The doctors taking care of you should be able to prevent and/or treat many of these problems with medicines and blood transfusions. This is sufficient for the majority of patients, until their blood cells begin to grow normally. If you do not receive a transplant after this treatment your own marrow would either not grow back at all or not grow back fast enough to prevent your death from bleeding and/or a lethal infection.

Graft Failure: The transplanted blood stem cells may fail to grow or be rejected by your body. The treatment you receive before your transplant has been designed to reduce the chance of rejection as much as possible. Recent measures used to prevent this problem have reduced the likelihood of rejection or early graft failure to less than 5%. If your donated stem cells fail to grow due to rejection, viral infection, or other causes, you may be offered a second transplant. This second transplant would be from the same donor. If your transplant fails and you could not receive a second transplant from your original donor, the result would probably be death except in the unlikely event that your own marrow recovered despite the high dose radiation treatment and chemotherapy. Second transplants are also used in rare cases to improve otherwise delayed recovery of normal immune function that helps you fight infections.

Graft-versus-host disease [GVHD]: GVHD is a disease in which the donor blood stem cells react against your own body organs and tissue. There are both early and late forms of this problem. GVHD may never appear, may be mild and temporary, or may lead to severe complications including death. Early GVHD can cause skin rash, diarrhea, or liver injury. In addition, late GVHD can also cause damage to many other organs including lungs and sexual organs. It may be severe enough to cause death in 15% of patients receiving non-T cell depleted transplants. Severe GVHD increases the chance of infections that may result in

death. Both forms of GVHD appear to occur less often in patients receiving T cell depleted transplants.

Low Number of Donor Blood Stem Cells: There is a small risk that the number of blood stem cells collected from your donor may not be enough cells for you to receive a fully T cell depleted peripheral blood stem cell transplant. A certain number of donor blood stem cells are necessary in order for the donor cells to start growing in your body. Your doctor can predict this based on tests done in the laboratory during the T cell depletion procedure. In such a situation, your doctor may decide it is in your best interest to receive a non-T cell depleted transplant. Should this occur, then you would receive medications to reduce the chances of developing GVHD. Medications are essential to prevent GVHD if you receive a transplant that has too many T cells.

Relapse of Leukemia: The leukemia may return at a later date even if the transplant is successful. Based on current information, this risk is lower than your chance of relapse or death from your disease without a transplant. The risk of relapse may be higher with a T cell depleted transplant compared to a conventional transplant, but we do not know this for sure.

Second Cancers: Anyone who has ever had cancer once is more likely to develop a second kind of cancer, than someone who has never had any cancer at all. You may develop a second type of cancer that is different from your leukemia because of the radiation and chemotherapy given for your transplant. Approximately 2% of patients alive 10 years and 7% alive 15 years after their transplant will have developed another cancer. Many of the second cancers can be successfully treated.

Sterility: The combination of radiation and chemotherapy given for the blood stem cell transplant will make you unable to have children [sterile]. This is usually permanent. Other sexual function, including the ability to have sexual relations, may also be affected. These side effects occur in non-T cell depleted transplants as well. If you want to maintain the possibility of having children later and are a man, ask for a referral to a sperm bank. The options for women are not as simple and may not be in your best interest due to the time required. Your doctor can discuss these with you and try to make referral. These options may or may not be feasible, depending on the type of chemotherapy that you have already received for your disease. Also, should any of this delay your transplant to an extent that would reduce your chances of success, your doctor will recommend that you proceed directly with the transplant.

Transfusion Risks: There are risks from transfusions of all blood products after transplant. These risks may include too much fluid, serious allergic reactions, and infections such as viral hepatitis B or C, cytomegalovirus infection, and AIDS. All blood products you receive will be screened against such diseases. The screening standards are set according to blood banking guidelines established by outside regulatory agencies and apply to all patients in this and other hospitals. Screening procedures and standards are constantly updated as new technology comes along. This helps to reduce the risks of transmitting disease by blood transfusions.

Potential Allergic Reactions to Murine Proteins: Mouse (murine) protein antibodies are used in the CliniMACS processing procedure. If you have a pre-existing allergy, you may be at risk for allergic reactions during infusion of the processed donor stem cells, although only very small amounts of mouse protein are present. To date, no allergic reactions are reported in patients receiving cells processed with the CliniMACS System. You should notify your physician if you have been told you have an allergy to mouse proteins or if you know that you have received a product containing mouse antibodies. Some patients that have previously been exposed to products containing mouse antibodies may develop their own antibodies against mouse proteins. These are called human anti-mouse antibodies or HAMA. The presence of HAMA may possibly make a person more likely to develop an allergic reaction to mouse proteins, but this is not proven. Unfortunately, there is no well accepted test for measuring HAMA and it is not known whether the presence of HAMA would let us know if you would have an allergic reaction to mouse proteins. Therefore, we will not be testing for HAMA in this study. In the event that you do have an allergic reaction, epinephrine (a drug used for cardiac arrest) and antihistamines (drugs used for allergic reactions) will be available at your bedside during the infusion.

Organ Damage: Damage to any of the major organs, including the brain, may occur. This can be caused by a number of things: high-dose radiation and chemotherapy, reaction to other drugs, other destructive processes such as infection, GVHD or a combination of these factors. Your doctors will use antibiotics and other medications to reduce the risk of this happening. Although severe organ damage may have a fatal outcome, less than 10% of people receiving a T cell depleted peripheral blood stem cell transplant die from this type of complication.

Risk of Infections: Your ability to fight infections in a normal way [normal immune function] may not occur until 1-2 years after your transplant. Until that time, you will be very susceptible to infection. The infections can include germs you catch from your family and friends. Infections can also come from things that are very common and around us all the time. These common things normally do not cause disease when you have a normal immune system. In order to reduce your risks of these infections, you will be asked to follow the guidelines that you will be taught before leaving the hospital. You will also be given certain medications where preventive treatment has been found to be effective. Unfortunately, even following all the guidelines and taking all the preventive medications does not guarantee that you will not develop a serious infection. If you get an infection you will require additional tests and treatment. You may need to return to the hospital. Many infections can be treated successfully, but some can lead to death in spite of hospitalization and treatment. Infections are a common problem for all transplants, but they might occur more often after a T cell depleted transplant.

Risk of Death: The risk of dying from a problem in the immediate period (approximately the first month) after your transplant is 5-10%. The risk of death during this period depends on many things. Things like your age, any other medical problems, or the amount of prior treatment you have had for leukemia effect your risk. The tests you have before your transplant are used by your doctor to see if you are at increased risk for serious complications including death from the transplant. We do not believe the risk of death will be higher with a T cell depleted transplant than with a non-T cell depleted transplant.

During the time you are in the study, you will be informed of any new findings that might affect your willingness to continue should they occur.

If you are injured as a result of your participation in this research study, emergency care, hospitalization, and outpatient care will be made available to you by [ ] and billed to you as part of your regular medical expenses. No money will be provided as compensation for a research-related injury.

As with any treatment, there may be yet unknown and/or unexpected side effects from a T cell depleted blood stem cell transplant.

10. What other alternatives or treatments are available if you do not want to be in this study?

Participation in this study is entirely voluntary. You are free to refuse to be in the study, and your refusal will not affect current or future health care you receive at this hospital. You and your doctor will discuss any other treatment options available to you including:

- No treatment
- Chemotherapy
- A transplant using your own bone marrow or peripheral blood stem cells
- A transplant of bone marrow or blood stem cells from a relative without T cell depletion
- A transplant of bone marrow, blood stem cells or cord blood cells from a donor who is not related to you
- A transplant of bone marrow or blood stem cells from a relative using T cell depletion at another institution that is not participating in this study

11. What are the possible benefits to you?

If a T cell depleted transplant proves to be more effective in reducing the risk of GVHD without increasing the incidence of other serious side effect, you may benefit by participating in this study. On the other hand, you may receive no direct benefits from this study. You may or may not benefit from the scheduled medical assessments required for this study, and extra support from personnel working for this study.

12. What are the possible benefits to others?

You may be helping other patients get better treatment in the future.

13. If you choose to take part in this study, will it cost you anything?

You and/or your insurance company will pay all medical expenses relating to, or arising from the blood stem cell transplant. You or your insurer will not be charged for the T cell depletion of the blood stem cell graft since this is considered research. You or your insurer will not be charged for samples and tests that are considered research. For questions about your costs, financial responsibilities, and/or medical insurance coverage for your transplant and this study, please contact /Center/ Financial Counselor at /Number/.

14. Will you be paid for taking part in this research study?
No.

15. What if you are injured because of the study?
If you are injured while taking part in this study, medical care will be provided at this center. No funds have been set aside to pay you if you are injured. You or your insurance company will be charged for ongoing medical care and/or hospitalization. Contact your doctor or one of the people listed at the start of this form if you are concerned about a research-related injury.

16. How can you withdraw from this research study?
You may decide to quit this study at any time, for any reason, without notice. However, if you quit after you have had some or all of the chemotherapy and radiation treatment but before your transplant is given, then your blood counts may not return and you could die. Even if you withdraw from the study after starting treatment, you will require medical follow-up to manage side effects of treatment you have received. If you decide to quit, we ask that you tell [the Principal Investigator] in writing (his/her address is on the front page of this form). You may also withdraw from the study by providing verbal notification to your physician and a witness. If you do take back your consent, there will be no penalty. You will not lose anything you are entitled to. You will continue to receive proper medical care. If you have any questions about your rights as a study subject, you may phone the Institutional Review Board (IRB) office at /number/.

17. If you quit the study, can information about you still be collected and used?
If you quit the study, we ask that you let us continue using all information that was already collected. We also ask that you let your doctor continue to tell us about your progress until two years after your transplant. You may say no at any time.

18. Can the Principal Investigator withdraw you from this research study?
You can be taken off the study (with or without your consent) for any of these reasons:

- Staying in the study would be harmful to you.
- You need treatment not allowed in this study.
- You do not follow directions.
- The study is cancelled.

19. How will your privacy and the confidentiality of your research records be protected?
The centers and doctors in charge of this study will keep your personal information as private as possible. They will do their best to see that it is shared only when required by state or federal law or the terms of this consent. It is impossible to promise total privacy. In addition to following state and federal law, the organizations listed below may read or copy your records to make sure the study information is correct. Your research and medical records will have your name on them. They will include things such as your medical history, results of your blood tests and exams, as well as reports about your treatment and office visits.

In order to understand the results of the study, people from the /Center Name/ and the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) will need to see medical records with your name on them. These people include:

- Doctors in the study
- Transplant center committees
- People (who are not doctors) who check the safety and progress of studies
- Members of the Institutional Review Board (this committee safe-guards the rights of persons taking part in research), and
- People from the government (the National Institutes of Health and the Food and Drug Administration)

Your research and medical records may be shown to these organizations:

- [Institution]
- Office of Human Research Protection (OHRP)

Information related to or resulting from your stem cell transplant will be reported to the Center for International Blood and Marrow Transplant Research (CIBMTR). The CIBMTR is a voluntary organization of basic and clinical scientists working together in an effort to gather information on results of stem cell and marrow transplants. This information is used to guide clinical decisions and identify ways to improve transplant outcomes. Scientific data or medical information (not identifiable with you) that could be useful to others may be presented at meetings and/or published in medical journals.

Data about your progress will be sent to the Blood and Marrow Clinical Trials Network Data Coordinating Center and the International Blood and Marrow Transplant Registry. Your name and other personal identifiers will NOT be sent to these organizations.

Summary data will be shared with Miltenyi, the company that is providing the CliniMACS materials to do the T cell depletion. They may also view your study record.

We will do all we can to keep your medical records private. Your name will not be used in any report of study results. Only study personnel will have access to your information. However, if any of your answers lead us to believe you are seriously depressed or in danger of hurting yourself, your doctor will be notified. For questions about access to your medical records, please contact /name / at/number/.

20. What is the expiration date for keeping your records?

Study records will be kept indefinitely by the transplant center for re-analysis and follow-up. If you have questions about the keeping of your research records or access to your files, please call /name/at /number/.

The data sent to the Blood and Marrow Clinical Trials Network Data Coordinating Center will be kept for 5 years after the study has ended.

21. How will the researcher(s) benefit from you being in this study?

The researchers have no money invested in this study. But, in general, presenting research results helps the career of a scientist. Therefore, the Principal Investigator may benefit if the results of this study are presented at scientific meetings or in the scientific press. In addition, the Principal Investigator is being paid a small amount to cover the cost of the study.

22. HIPAA[1] authorization to use and disclose individual health information for research purposes

a. Purpose: As a research participant, I authorize the Principal Investigator and the researcher's staff to use and disclose my individual health information for the purpose of conducting the research study entitled *A Single Arm, Multicenter Phase II Trial of Transplants of HLA-Matched, CD34$^+$ Enriched, T cell Depleted Peripheral Blood Stem Cells Isolated by the CliniMACS System in the Treatment of Patients with AML in First or Second Morphological Complete Remission*.

b. Individual Health Information to be Used or Disclosed: My individual health information that may be used or disclosed to conduct this research includes: demographic information (e.g., age, date of birth, sex, weight), medical history (e.g., diagnosis, complications with prior treatment), physical examination findings, and laboratory test results obtained at the time of work-up and after transplantation (e.g., bone marrow tests, blood tests, biopsy results).

c. Parties Who May Disclose My Individual Health Information: The researcher and the researcher's staff may obtain my individual health information from (*list hospitals, clinics or providers from which health care information can be requested*).

_____

_____

_____

d. Parties Who May Receive or Use My Individual Health Information: The individual health information disclosed by parties listed in item "c." above and information disclosed by me during the course of the research may be received and used by the following parties:

- Principal Investigator and the researcher's staff
- Dr. Steven Devine, Study Chairperson and staff/laboratories at Ohio State University
- Dr. Richard O'Reilly, Study Chairperson and staff/laboratories at Memorial Sloan-Kettering Cancer Center
- National Heart, Lung and Blood Institute (NHLBI) and National Cancer Institute (NCI), both of the National Institutes of Health (NIH), study sponsors
- Blood and Marrow Transplant Clinical Trials Network (BMT CTN), data coordinating center

[1] HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information.

- U.S. government agencies that are responsible for overseeing research such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP)
- U.S. government agencies that are responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments

e. Right to Refuse to Sign this Authorization: I do not have to sign this Authorization. If I decide not to sign the Authorization, I will not be allowed to participate in this study or receive any research-related treatment that is provided through the study. However, my decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.

f. Right to Revoke: I can change my mind and withdraw this authorization at any time by sending a written notice to the Principal Investigator to inform the researcher of my decision. If I withdraw this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about me will be collected by or disclosed to the researcher for this study.

g. Potential for Re-disclosure: My individual health information disclosed under this authorization may be subject to re-disclosure outside the research study and no longer protected. Examples include potential disclosures for law enforcement purposes, mandated reporting or abuse or neglect, judicial proceedings, health oversight activities and public health measures.

h. This authorization does not have an expiration date.

23. Subject's Consent

I have been informed of this study's purpose, procedures, possible benefits and risks. I have been given a chance to ask questions and have had them answered to my satisfaction. I understand that I can ask more questions at any time.

I voluntarily agree to participate in this study.

By signing this consent form, I have not given up any of the legal rights, which I otherwise would have as a subject in a research study.

_____ _____
Signature of Subject Date

_____
Print Name of Subject

Certification of Counseling Healthcare Professional

I certify that the nature and purpose, the potential benefits, and possible risks associated with participation in this study have been explained to the above individual and that any questions about this information have been answered.

_____ _____
Signature of Counseling Healthcare Professional Date

_____
Print Name of Counseling Healthcare Professional

APPENDIX B-2

DONOR INFORMED CONSENT

Donor Informed Consent to Participate in Research

This is a consent form for a research study. This form is to help you decide if you want to participate in this study.

Your family member has acute leukemia and may be treated with a blood stem cell transplant using blood stem cells donated by a family member like you. This type of transplant is called a peripheral blood stem cell transplant. The goal of this study is to see if transplant patients have better results when the cells that can cause a serious complication called graft-versus-host disease (GVHD) are removed from the donor's stem cells prior to transplantation.

This consent form tells you about the study. The doctors in charge of this study (the investigators) or other staff will also discuss this study with you and answer any questions you might have. Before you decide to join this study, please read this information and ask any questions about things you do not understand. Some people find it helpful to have a family member or friend with them to help ask questions and listen to information.

This study will give more information to doctors about future treatment choices for patients with leukemia. It is important to know that:

- You will not be paid to be in this study.
- You, your medical insurance company or the patient's medical insurance company will pay for all medical bills for your treatment.
- You will not be charged for research tests – tests you would not normally have if you were not a part of this study.

Before you decide to join the study, please read the information below. Feel free to ask questions to understand your rights. It is your choice to take part in this study.

1. Title of Research Study

A Single Arm, Multicenter Phase II Trial of Transplants of HLA-Matched, $CD34^+$ Enriched, T cell Depleted Peripheral Blood Stem Cells Isolated by the CliniMACS System in the Treatment of Patients with AML in First or Second Complete Remission

2. Principal Investigator Contact Information at your Institution

Name/Title/Phone number/

3. Contact information for emergencies after hours or on weekends or holidays:
Name/Phone number/

4. Sponsors and Source of Funding or Other Material Support
This research study is paid for by the National Institutes of Health (NIH). The Blood and Marrow Transplant Clinical Trials Network (BMT CTN) will direct the research study. A company called Miltenyi will provide the CliniMACS system for doing the T cell depletion. This study will be done at many different medical centers, including [Center Name/Location].

5. What will be different for you as a donor of peripheral blood stem cells if you choose to participate in this study?
If you plan to donate blood stem cells to the patient, participating in this study does not change how you will donate blood stem cells. The donation process for you is the same for this study as it would be if the patient was receiving a standard transplant.

6. What is the purpose of this study?
The purpose of this study is to determine if radiation therapy and chemotherapy followed by transplantation of T cell depleted stem cells donated by you can effectively treat your family member's acute leukemia. The research portion of this study involves the process of removing of the T cells from your stem cells (T cell depletion) since this process is not routinely performed.

7. What will be done if you take part in this research study?
In order to determine whether you are medically able to donate blood stem cells you will have a complete medical history and physical and some laboratory tests will be performed. This physical will include an EKG (tracing of your heartbeat), blood tests (approximately 1-2 tablespoons), urine test and a pregnancy test (if female). These are standard tests to evaluate a person who may be a blood stem cell donor.

Granulocyte Colony Stimulating Factor (G-CSF) Injections
If the tests indicate you are eligible to be a stem cell donor, you will receive a drug called G-CSF. G-CSF is normally made by the body to help the bone marrow make white blood cells that fight infection. When given in larger doses than normally found in the body, G-CSF helps the cells needed for a transplant move from the bone marrow to the bloodstream. G-CSF is sold under the name Neupogen.

G-CSF is given as an injection under the skin. The first time you receive G-CSF, it will be given to you at the clinic. For the remaining shots, you may learn how to give them to yourself, have a family member or friend learn how to do it, have a visiting nurse do it or return to the clinic for them on a daily basis. You will continue to receive daily G-CSF shots under the skin for between five and seven days. You will be asked to return to the clinic on the morning of the 5th day of G-CSF treatment to start collecting your blood stem cells.

Apheresis
Blood stem cells are collected using a process called apheresis. Apheresis involves placing 2 catheters into blood veins in your arms. These catheters are then connected to the apheresis machine. The apheresis machine takes blood from your body, removes the stem cells from your blood, and returns the rest of the blood back to you. The apheresis procedure is done daily until enough stem cells are collected. This may involve up to three days of collections. Each session will last approximately 6 hours. During the session you will be lying down in a reclining chair. Since blood is being drawn through the catheter, there will be no additional needle sticks. However, if the catheter becomes clogged or otherwise fails to work, another catheter will be started. Your blood counts will be monitored during the process. Apheresis will be stopped if it is felt that continuing would harm your health.

Usually, the apheresis procedure can be done by inserting catheters into your veins as described above. However, if your veins are too small you may need to have a special catheter placed in a large blood vessel in your neck or chest. This is a routine procedure. It will be done in an operating or procedure room on an outpatient basis. A specially trained surgeon or radiologist will do the procedure.

At the end of two days, we will know if we have enough stem cells. If not, you will need to undergo one more apheresis procedure

If your family member fails to recover blood counts within a reasonable period of time after transplant, you may be asked to donate more blood stem cells or bone marrow. Bone marrow collection is done in the operating room. It will be explained to you in detail if it becomes necessary.

T Cell Depletion
The blood cells collected from you will have the T cells removed by a process called T cell depletion. The device used to do this is called CliniMACS. CliniMACS is an experimental device. This means it is not yet approved by the US Food and Drug Administration.

8. How long will I be in the study?
You will be in the study for up to several months from the time you sign the consent until approximately one month after stem cell collection. The actual process of taking G-CSF and then collecting your stem cells though takes less than a week. You will be contacted by phone approximately 30 days after initiation of G-CSF. You will be asked to answer questions about your health since your stem cells were collected.

9. Will you provide blood samples for research?
You will not be asked to provide blood samples for research.

10. What are the possible discomforts and risks?
Central Venous Catheter (Central line): If catheters cannot be placed in the blood veins in your arms, a central venous catheter will be needed. When a central venous catheter is put into one of the large veins in your chest, it may cause bleeding or infection. Rarely, one of your lungs could collapse. If this happens, another tube will be put into your chest until the

lung is fully re-expanded. While you have a central line in place, you have an increased chance of infection around it or in your blood. If this occurs, it will be treated with antibiotics. In some cases, the catheter may need to be removed and replaced with another catheter. Rarely, a blood clot can form on the tip of the catheter, break off, and go into the lungs (pulmonary embolus), which could cause shortness of breath and pain. This is very unlikely but if it did occur, your doctors may need to treat you with blood thinning medication.

Blood Drawing: You may experience discomfort, swelling, bruising and or bleeding at the site of the needle insertion. Less common side effects include dizziness, infection at the site of the catheter or feeling faint.

G-CSF: You will likely experience bone pain, feelings of tiredness, muscle aches, and headache. Less common side effects include low-grade fever, chills, and skin rash. Rarely, shortness of breath, wheezing, low blood pressure, and increased liver function tests occur. In extremely rare cases, rupture of the spleen has been reported following G-CSF treatment.

Leukapheresis (Apheresis): Apheresis is a procedure routinely used to collect platelets and other blood products from volunteer donors. However, rarely (less than 1% chance) side effects occur and include high or low blood pressure, muscle cramping, chills and fever, loss of red blood cells leading to anemia, and loss of platelets which may lead to easy bruising and bleeding. Transfusions of red blood cells and/or platelets may be necessary (less than 1% chance). The medicine used to prevent blood from clotting in the machine can cause tingling or numbness around your mouth, feet or hands and in rare cases bleeding. If these symptoms occur they go away quickly. You will be monitored closely during apheresis.

Participation in this study may cause some or all of the side effects listed above. Some of the side effects, if serious enough, may cause death. However, the risk of death is very small. The investigator is willing to discuss any questions you might have about the severity, frequency, and duration of these risks and discomforts.

Breach of Confidentiality: Medical records are considered confidential. These records are kept in a secured area accessible to people involved in the conduct of the study. You will not be identified by name in any publication or presentation of the results of this study. All data entered into a computer will be coded. No data that may be linked to you will be entered on any network computer that could allow access to confidential information. The master list will be stored off-line and available only to the principal investigator and his or her designee(s). Although we will make every effort possible to maintain confidentiality, there is however, a slight risk of loss of confidentiality.

11. As with any treatment, there may be yet unknown and/or unexpected side effects from donating peripheral blood stem cells.

Donating blood stem cells is routinely done and is not considered research. Unanticipated side effects may occur that have not been previously reported. If you have any unusual symptoms, you should report them immediately to your doctor.

In an attempt to avoid side effects, your doctor will examine you and obtain laboratory test (blood tests, chest x-ray, etc.) to determine the effects of the treatment and alter the drug doses if necessary.

12. What other alternatives are available if you do not want to be in this study?

Your participation is voluntary and you may choose not to participate in this research study or withdraw your consent at any time. Your choice will not at any time affect the commitment of your health care providers to provide care to you or the patient. There will be no penalty or loss of benefits to which you or your relative are otherwise entitled. Alternatives to participating in this research include donating your bone marrow to your relative, donating your blood stem cells for a transplant that is not part of this research study, or deciding not to donate either your bone marrow or blood stem cells. The investigator can discuss with you the other treatment options available to the patient should you decide not be a donor.

13. What are the possible benefits to you?

You will not benefit directly from participating in this research. You may receive indirect benefit from knowing that you may be helping your family member or other donors and patients in the future.

14. What are the possible benefits to others?

You may be helping other patients get better treatment in the future.

15. If you choose to take part in this study, will it cost you anything?

Normally the insurance company of the patient covers the medical expenses associated with collecting your blood stem cells. This will be reviewed with the patient's insurance company prior to collecting your stem cells. Neither you nor the insurance company will be charged for the T cell depletion of the peripheral blood stem cell graft since this is considered research. You will not be reimbursed for any direct or indirect personal expenses related to participation in the study. For questions about your costs, financial responsibilities, medical insurance coverage, donation, and/or this study, please contact /Center/ Financial Counselor at /Number/.

16. Will you be paid for taking part in this research study?

No.

17. What if you are injured because of the study?

If you are injured or become ill while taking part in this study, medical care will be provided at this center. No funds have been set aside to pay you if you are injured. You, your insurance company or the patient's insurance company will be charged for ongoing medical care and/or hospitalization. Contact your doctor or one of the people listed at the start of this form if you are concerned about a research-related injury.

18. How can you withdraw from this research study?

You may decide to quit this study at any time, for any reason, without notice. If you decide to quit, we ask that you tell [the Principal Investigator] in writing (his/her address is on the front page of this form). You may also withdraw from the study by providing verbal notification to

your physician and a witness. If you do take back your consent, there will be no penalty. You will not lose anything for which you are entitled. You will continue to receive proper medical care. If you withdraw from the study and do not donate either peripheral blood stem cells or bone marrow for your brother or sister after they have received their chemotherapy and radiation in preparation for the transplant, they will likely die from irreversible bone marrow damage.

If you have any questions about your rights as a study subject, you may phone the Institutional Review Board (IRB) office at /number/.

19. If you quit the study, can information about you still be collected and used?

If you quit the study, we ask that you let us continue using all information that was already collected. You may say no at any time.

20. Can the Principal Investigator withdraw you from this research study?

You can be taken off the study (with or without your consent) for any of these reasons:

- Staying in the study would be harmful to you.
- You need treatment not allowed in this study.
- You do not follow directions.
- The study is cancelled.

21. How will your privacy and the confidentiality of your research records be protected?

The centers and doctors in charge of this study will keep your personal information as private as possible. They will do their best to see that it is shared only when required by state or federal law or the terms of this consent. It is impossible to promise total privacy. In addition to following state and federal law, the organizations listed below may read or copy your records to make sure the study information is correct. Your research and medical records will have your name on them. They will include things such as your medical history, results of your blood tests and exams, as well as reports about your treatment and office visits.

In order to understand the results of the study, people from the /Center Name/, and the Blood and Marrow Transplant Clinical Trials Network (BMT CTN) will need to see medical records with your name on them. These people include:

- Doctors in the study
- Transplant center committees
- People (who are not doctors) who check the safety and progress of studies
- Members of the Institutional Review Board (this committee safe-guards the rights of persons taking part in research), and
- People from the government (the National Institutes of Health and the Food and Drug Administration) might also need to see medical records with your name on them.

Your research and medical records may be shown to these organizations:

- [Institution]
- Office of Human Research Protection (OHRP)

We will do all we can to keep your medical records private. Your name will not be used in any report of study results. Only study personnel will have access to your information. However, if any of your answers lead us to believe you are seriously depressed or in danger of hurting yourself, your physician will be notified. For questions about access to your medical records, please contact /name / at/number/.

22. What is the expiration date for keeping your records?

Study records will be kept indefinitely by the transplant center for re-analysis and follow-up. The information sent to the Blood and Marrow Clinical Trials Network will be kept for five years following the closure of the study. If you have questions about the keeping of your research records or access to your files, please call /name/at /number/.

23. How will the researcher(s) benefit from you being in this study?

The researchers have no money invested in this study. But, in general, presenting research results helps the career of a scientist. Therefore, the Principal Investigator may benefit if the results of this study are presented at scientific meetings or in the scientific press. In addition, the Principal Investigator is being paid a small amount to cover the cost of the study.

24. HIPAA[1] authorization to use and disclose individual health information for research purposes

a. Purpose: As a research participant, I authorize the Principal Investigator and the researcher's staff to use and disclose my individual health information for the purpose of conducting the research study entitled *A Single Arm, Multicenter Phase II Trial of Transplants of HLA-Matched, CD34$^+$ Enriched, T cell Depleted Peripheral Blood Stem Cells Isolated by the CliniMACS System in the Treatment of Patients with AML in First or Second Morphological Complete Remission*.

b. Individual Health Information to be Used or Disclosed: My individual health information that may be used or disclosed to conduct this research includes: demographic information (e.g., age, date of birth, sex, weight), medical history (e.g., diagnosis, complications with prior treatment) and physical examination findings.

c. Parties Who May Disclose My Individual Health Information: The researcher and the researcher's staff may obtain my individual health information from (*list hospitals, clinics or providers from which health care information can be requested*).

_____

_____

_____

[1] HIPAA is the Health Insurance Portability and Accountability Act of 1996, a federal law related to privacy of health information.

d. Parties Who May Receive or Use My Individual Health Information: The individual health information disclosed by parties listed in item "c." above and information disclosed by me during the course of the research may be received and used by the following parties:

- Principal Investigator and the researcher's staff
- Dr. Steven Devine, Study Chairperson and staff/laboratories at Ohio State University
- Dr. Richard O'Reilly, Study Chairperson and staff/laboratories at Memorial Sloan-Kettering Cancer Center
- National Heart, Lung and Blood Institute (NHLBI) and National Cancer Institute (NCI), both of the National Institutes of Health (NIH), study sponsors
- Blood and Marrow Transplant Clinical Trials Network (BMT CTN), data coordinating center
- U.S. government agencies that are responsible for overseeing research such as the Food and Drug Administration (FDA) and the Office of Human Research Protections (OHRP)
- U.S. government agencies that are responsible for overseeing public health concerns such as the Centers for Disease Control (CDC) and federal, state and local health departments

e. Right to Refuse to Sign this Authorization: I do not have to sign this Authorization. If I decide not to sign the Authorization, I will not be allowed to participate in this study or receive any research-related treatment that is provided through the study. However, my decision not to sign this authorization will not affect any other treatment, payment, or enrollment in health plans or eligibility for benefits.

f. Right to Revoke: I can change my mind and withdraw this authorization at any time by sending a written notice to the Principal Investigator to inform the researcher of my decision. If I withdraw this authorization, the researcher may only use and disclose the protected health information already collected for this research study. No further health information about me will be collected by or disclosed to the researcher for this study.

g. Potential for Re-disclosure: My individual health information disclosed under this authorization may be subject to re-disclosure outside the research study and no longer protected. Examples include potential disclosures for law enforcement purposes, mandated reporting or abuse or neglect, judicial proceedings, health oversight activities and public health measures.

h. This authorization does not have an expiration date.

25. Donor's Consent

I have been informed of this study's purpose, procedures, possible benefits and risks. I have been given a chance to ask questions and have had them answered to my satisfaction. I understand that I can ask more questions at any time.

I voluntarily agree to participate in this study.

By signing this consent form, I have not given up any of the legal rights, which I otherwise would have as a subject in a research study.

_____ _____
Signature of Donor Date

_____
Print Name of Donor

Certification of Counseling Healthcare Professional

I certify that the nature and purpose, the potential benefits, and possible risks associated with participation in this study have been explained to the above individual and that any questions about this information have been answered.

_____ _____
Signature of Counseling Healthcare Professional Date

_____
Print Name of Counseling Healthcare Professional

APPENDIX B-3

DONOR ASSENT

Donor Informed Assent to Participate in Research

This is a form for a research study. This form is to help you decide if you want to participate in this study.

Purpose of the Research Study
Your family member has acute leukemia and may be treated with a transplant of peripheral blood stem cells from a matched family member. The goal of the study is to see if patients have better results using a peripheral blood stem cell transplant in which the cells that can cause a serious complication called graft versus host disease (GVHD), are removed from the donor's peripheral blood stem cells prior to transplantation.

You are being asked to be in the study because you are a match for your brother or sister and can donate peripheral blood stem cells to them. Joining this study does not change how you will donate peripheral blood stem cells for your brother or sister. Your doctor or another person on the study team will explain to you what you must do if you are going to donate peripheral blood stem cells for your brother or sister. The team will also follow you closely to see if you are having any side effects while donating peripheral blood stem cells on the study.

If you have any questions, ask your doctors and make sure you understand their answers.

Your parents (or a guardian) are also asked for their permission for you to join this treatment study.

I agree to donate peripheral blood stem cells in this study.

_____ _____
Signature of Donor Date

_____
Print Name of Donor

_____ _____
Signature of Doctor Date

_____
Print Name of Doctor

APPENDIX C

LABORATORY PROCEDURES

APPENDIX C

Laboratory Procedures

1. HLA TYPING

Patient HLA testing and evaluation must be completed within four weeks prior to the initiation of conditioning therapy. All donors registered in the study will have HLA testing and evaluation completed within 28 days prior to the day of transplantation.

HLA typing can be done by either serologic or DNA methods for HLA-A, -B and –C; and low resolution DNA method for DRB1 consistent with NMDP standard procedures.

2. CHIMERISM

Samples of peripheral blood are collected for chimerism studies according to institutional standards. An initial pre-transplant peripheral blood sample is collected four weeks prior to the initiation of conditioning therapy to identify informative genetic differences between patient and donor for subsequent analyses. Additional peripheral blood samples are collected post-transplant for T-lymphoid and myeloid lineage specific chimerism studies on Day 28, 100, 180 and 365.

Baseline donor samples are collected within 28 days of transplantation for chimerism studies. DNA studies are required for sex-matched donor/recipient pairs. For sex mismatched donor/recipient pairs, cytogenetic studies or FISH are acceptable.

3. PATHOLOGY/CYTOGENETICS STUDIES

A bone marrow biopsy/aspirate is required within four weeks prior to the initiation of conditioning therapy for confirmation of remission status. Assessment of relapse of disease is mandatory on Day 100, 180 and 365 post-transplant using bone marrow aspirate/biopsy.

Pathology and cytogenetic studies will be conducted per institutional guidelines.

4. FLOW CYTOMETRY

In addition to final product viability, $CD3^+$ T cell content and $CD34^+$ cell content, the following parameters will be measured for each CD34-enrichment procedure:

Starting HPC-A Product
- TNC
- Viability (7-AAD method)
- $CD3^+$ T cell content
- $CD34^+$ cell content

Pre-Selection, Post-Platelet Wash and Antibody Wash
- TNC
- Viability
- $CD34^+$ cell content

Final Product
- TNC
- $CD3^+CD4^+$ T cells
- $CD3^+CD8^+$ T cells
- $CD3\text{-}CD56^+CD16^+$ NK cells
- $CD19^+$ or $CD20^+$ B cells
- $CD14^+$ monocytes

Flow cytometry will be done in keeping with the BMT CTN 0303 protocol SOPs for Product Processing and the BMT CTN MOP.

5. IMMUNE RECONSTITUTION

Samples of heparinized peripheral blood (5 mL) will be obtained from patients at 1, 3, 6 and 12 months post-transplant. Aliquots of peripheral blood will be stained with the panels described in Table C.1 and the percentage of nucleated cells with each phenotype determined using standard Cell-Quest (or similar) analysis templates. The transplant center will send the coordinating center the results from a CBC with differential performed on the sample as well as the calculated numbers of cells/mcL of each phenotype as determined by the results of the FACS analysis. The transplant center will calculate the absolute numbers of subsets of immune cells in the blood based upon the absolute number of leukocytes in the blood and the percentage of leukocytes that are T, B, or NK cell subsets as defined by multi-parameter flow cytometry.

TABLE C.1: FLOW CYTOMETRY FOR IMMUNE RECONSTITUTION

Tube #	Class of Cells	Cell Types	Measured Blood Cell Subsets	Antibodies
1	Lymphoid Subsets	T cells	$CD8^+$	anti-CD3 (FITC) anti-CD8 (PE) anti-CD45 (PerCP)
2	Lymphoid Subsets	T cells	$CD4^+$	anti-CD3 (FITC) anti-CD4 (PE) anti-CD45 (PerCP)
3	Lymphoid Subsets	NK cells	$CD56^+$ $CD16^+$ $CD3^-$	anti-CD3 (FITC) anti-CD56 + anti-CD16 (PE) anti-CD45 (PerCP)
4	Lymphoid Subsets	B-cells	$CD5^+$ $CD19^+$ B cells $CD5^+$ $CD19^+$ Immature B cells	anti-CD5 (FITC) anti-CD19 (PE) anti-$CD45^+$ (PerCP)
5	Gating controls	Monocytes	CD14+ cells	anti-IgG1 isotype control anti-CD14 (PE) anti-$CD45^+$ (PerCP))
6	Isotype Controls	Isotype Ig	Non-specific staining	irrelevant (FITC) (PE) (PerCP) conjugates to murine monoclonal Ab

6. IMMUNOGLOBULIN MONITORING

Quantitative immunoglobulin levels including IgG, IgA and IgM will be determined within four weeks prior to the initiation of conditioning therapy and on Day 63, 100, 180 and 365 post-transplant. Testing will be done in keeping with the BMT CTN MOP and local institutional practice.

7. EPSTEIN-BARR VIRUS (EBV) SURVEILLANCE

Epstein-Barr Virus (EBV) surveillance using a real time quantitative EBV DNA PCR plasma-based assay will be performed weekly post-transplant until Day +100 and then at least monthly until Day +180. Peripheral blood samples (5 mL) containing EDTA anticoagulant will be collected and sent to the central laboratory at the University of Washington. Samples will be shipped cooled, within 24 hours of collection, using insulated shipping kits containing frozen gel packs to the University of Washington laboratory via priority overnight FedEx service for receipt by 10:30am PT, the next day. If the laboratory will not receive the sample(s) within 24 hours of collection, the plasma/serum must be separated within 24 hours and the plasma is to be shipped frozen in a cryovial on dry ice. Samples will be shipped in compliance with the shipping procedures specified in the BMT CTN 0303 Laboratory Sample Information Guide. The University of Washington should report all test results to the Transplant Center within 2 business days from the receipt of sample.

8. LABORATORY SPECIMEN COLLECTION, STORAGE AND SHIPPING PROCEDURES

Standard procedures for collection, storage, and shipping of specimens will be followed according to the NMDP and the NHLBI guidelines. Samples will be given a unique alphanumeric code that contains no personal identifiers. Transplant Center Coordinators will hold the link to the code. Laboratory staff will not have access to the link.

9. LABORATORY CONTRACTS AND REMAINING SAMPLES

All laboratory studies will be performed at laboratories under contract with the NMDP on behalf of the BMT CTN. The laboratory contract specifies that any remaining serum must be disposed of after completion of testing and results have been verified.

TABLE C-2: SCHEDULE OF LABORATORY EVALUATIONS

	TYPE OF SAMPLE	DATES SAMPLES OBTAINED	STORAGE	SHIPPING SPECIFICATIONS	LOCATION OF TEST PERFORMED
HLA Typing	According to institutional practice	*Patient*: Within four weeks prior to the initiation of conditioning therapy. *Donor*: Within 28 days prior to the day of transplantation.	According to institutional practice	N/A	Transplant Center
Chimerism	According to institutional practice	*Patient*: Within four weeks prior to the initiation of conditioning therapy and on Day 28, 100, 180 and 365 post-transplant. *Donor*: Within 28 days prior to the day of transplantation.	According to institutional practice	N/A	Transplant Center
Pathology/ Cytogenetic Studies	According to institutional practice	Within four weeks prior to the initiation of conditioning therapy and on Day 100, 180 and 365 post-transplant	According to institutional practice	N/A	Transplant Center
Flow Cytometry	According to institutional practice	Obtained from each leukapheresis product pre- and post-$CD34^+$ selection.	Store according to SOPs for Product Processing and institutional practice	N/A	Transplant Center
Immune Reconstitution	5 mL heparinized peripheral blood	1, 3, 6 and 12 months post-transplant.	According to institutional practice	N/A	Transplant Center
Immunoglobulin Monitoring	According to institutional practice	Within four weeks prior to the initiation of conditioning therapy and on Day 63, 100, 180 and 365 post-transplant.	According to institutional practice	N/A	Transplant Center

	TYPE OF SAMPLE	DATES SAMPLES OBTAINED	STORAGE	SHIPPING SPECIFICATIONS	LOCATION OF TEST PERFORMED
Epstein-Barr Virus (EBV) Surveillance	5 mL peripheral whole blood samples (EDTA lavender top tube)	Weekly post-transplant until Day +100 and then at least monthly until Day +180.	***If laboratory will receive sample(s) within 24 hours of collection,*** store cooled (4°C) until shipped.	Ship cooled (4°C) within 24 hours of collection using insulated shipping kits containing frozen gel packs via priority overnight service.	University of Washington laboratory
			If laboratory will not receive sample(s) within 24 hours of collection, separate plasma/serum and freeze plasma in cryovial until shipped.	Ship frozen in cryovial on dry ice real-time via priority overnight service.	

APPENDIX D

HUMAN SUBJECTS

APPENDIX D

Human Subjects

Subject consent: Candidates for the study will be identified as described in Chapter 4 of the protocol. The Principal Investigator or his/her designee at each transplant center will contact the candidates and enroll them onto the study. The study coordinator or physician at each center will provide the patient with information about the purpose of the study and obtain consent. The Network will provide a template of the consent form to each center. Each center will customize the template according to their local requirements and submit it for review by the local Institutional Review Board (IRB). Each center must provide evidence of IRB approval. The Data Coordinating Center of the BMT CTN will review and approve the modified consent form.

Confidentiality: Confidentiality will be maintained by individual names being masked and assigned a patient identifier code. The code relaying the patient's identity with the ID code will be kept separately at the center. The ID code will be transmitted to the network.

Participation of women and minorities, children and other populations: Women and ethnic minorities will be included in this study. Children (age < 18) are not eligible for this protocol for the following reasons:

1. The results with regards to acute and chronic GVHD with HLA-identical sibling donors in children are too good to warrant entry onto this trial.

2. As the results in children are generally quite good, the exposure of other minors as donors to G-CSF and leukapheresis, that requires a central venous catheter, is not justifiable.

3. Initial data suggests that allogeneic peripheral blood stem cell transplants in children results in poorer outcomes than allogeneic bone marrow transplants. This further argues against testing this strategy in a good-risk, pediatric population.

Accrual will be monitored within each center with the expectation that the enrolled patient population is representative of the transplanted patient population at each center. Representation will be examined by comparing gender, race, ethnicity and age distributions. Accrual of minority patients will be expected to be in proportion to the number of minority patients transplanted at each center. The DCC and NHLBI will discuss enrollment anomalies with the centers.

APPENDIX E

MEMORIAL SLOAN-KETTERING STANDARD OPERATING PROCEDURES FOR HYPERFRACTIONATED TOTAL BODY IRRADIATION

APPENDIX E

Memorial Sloan-Kettering Hyperfractionated TBI Standard Operating Procedures

Patients are treated isocentrically (at 440 cm) in the standing position (AP/PA) using a specially-designed stand with a built-in ion chamber for in-vivo dosimetry. Lung blocks are placed on the stand in close proximity to the patient surface and the position of the lung blocks is verified prior to the first treatment. The lung block thickness is such that the dose at a depth of 10 cm is approximately 50% of prescription. A dose of 1375 cGy is delivered in 11 fractions with 15 MV x-rays at a dose rate of 8-13 cGy/min as calculated to mid-plane pelvis. The gantry is rotated to 275º with the collimator being set to the largest field size and rotated to an angle of 45º. A 1.0 cm Lexan spoiler is placed in front of the stand approximately 10-20 cm from the patient to ensure 90-95% skin dose. In-vivo dosimetry is done for each fraction with a tolerance of +/-5% of the fractional prescription dose.

On the final two days of treatment, anterior and posterior electron boosts are delivered to the portion of the chest wall, which was shielded by lung blocks. The patient is treated in a sitting position with the gantry at 90º and at a SSD of 97-105 cm. A dose of 600 cGy is delivered in two fractions to the depth of the chest wall-lung interface as measured on a CT scan. For male patients, a testicular electron boost of 400 cGy may be given in one fraction. The patient is treated in the recumbent position with a polystyrene and lead-backed shield placed under the testes to minimize the dose to the rectal area. The gantry is angled to achieve skin apposition.

APPENDIX F

CHEST WALL BOOST PROCEDURES

APPENDIX F

Procedure for FTBI Chest Wall Boost Using the CMS Treatment Planning System

Electron energy to the chest wall is calculated, to the CT slice **which is 2 cm inferior to the central slice of the electron field** (2/3 down the mean height of both lungs) such that the 90% isodose level is at the pleural surface, while the 20% isodose lines from the parallel opposed portals are not touching.

The treatment planning shall be performed *on the central axis slice*, where the source to surface distance (SSD) is 100 cm to the chest wall. This matches the treatment arrangement for the chest wall boost. However, the electron energies and beam weighting shall be calculated to an off axis slice 2 cm inferior central axis (this is the slice 2/3 down the mean height of both lungs) known as the *planning CT slice*. The *planning CT slice* will receive 300 cGy to the pleural surface and the 20% isodose lines from the parallel-opposed beams will not be touching.

LUNG BLOCKS AND PATIENT MIDLINE

1. Draw in the lung blocks on the AP film. Blocks are drawn with a 2-cm margin from the diaphragm, 2 cm margin from the vertebral edge, and a 1.5 cm margin from the most lateral margin of the rib cage.
2. Draw in the patient's midline on the diagnostic X-ray film. The line should be the mid-distance between the vertebral edge blocks.
3. The physicist should draw the patient's midline on the film when selecting the **central axis and the planning** CT slices.
4. Select the central axis and the planning CT slices for external contouring.

Weight the beams individually in order to deliver 300 cGy to each interest point at the pleural surface of the planning slice. Use the following formula to calculate the weighting:

$$\text{Weight (in cGy at dmax)} = \frac{300 \text{ cGy}}{\text{Interest Point dose at the pleural surface of the planning slice}}$$

APPENDIX G

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APPENDIX G

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