CLINICAL RESEARCH PROJECT

Protocol #13-H-0144
IDE 15632
Product: Miltenyi CliniMACS® CD34Selection System
IDE Holder: A. John Barrett, M.D.

Date: April 12, 2018

To: Richard Cannon, MD, Chair, NHLBI IRB

Title: Peripheral blood stem cell allotransplantation for hematological malignancies using ex vivo CD34 selection – a platform for adoptive cellular therapies.

Other identifying words: AML, ALL, CLL, CML, MDS, MPD, NHL

Principal Investigator:

*Sawa Ito, M.D. H.B., NHLBI (E)	326-5233	Bldg 10, CRC 3-5330

Medically Responsible Investigator:

*Richard Childs, MD, HB, NHLBI (E)	594-8008	Bldg 10, CRC 3-5332

Associated Investigators:

David Stroncek, M.D., DTM, Clinical Center (E)	496-9702	Bldg 10, 1C7111
Pawel Muranski, MD, NHLBI (V)	240-688-3538	Columbia University, NY
Deborah Citrin, MD, ROB, NCI (E)	496-5457	Bldg 10, CRC 3B43C
Eleftheria Koklanaris, RN, Research Nurse, HB, NHLBI (E)	594-4709	Bldg 10, CRC 3-2473
Colin Wu, PhD, Biostatistician, DECA/OBR, NHLBI (E)	435-0440	RKL2, Room 8218
Xin Tian, PhD, Biostatistician, DECA/OBR/NHLBI (E)	435-1298	RKL2, Room 8217
A. John Barrett, M.D., M.S. HB, NHLBI (V)	402-4170	Bldg 10, CRC 3-5322

Note: **Investigators designated to obtain informed consent from research subjects.*

Collaborators:
Catherine Bollard, MD, Children's National Medical Center, Washington DC
Kim Green, PhD, NIAD

Subjects in Study:	Number	Sex	Age range
Subjects:	up to 192 (recipients & donors)	either	Recipients: 10-80 inclusive Donors: > 2 - <80

Project involves ionizing radiation?	Yes (medically indicated)
Off-site project?	No
Multi-institutional project?	No
DSMB Involved?	Yes
Tech Transfer?	Yes

PRECIS

Peripheral blood stem cell transplant research carried out by the NHLBI BMT Unit focus on transplant techniques designed to decrease graft versus host disease (GVHD), increase the graft-versus-leukemia (GVL) effect and reduce the risk of post-transplant graft rejection.

Through incremental transplant clinical trials we have shown that by controlling the stem cell (CD34+ cell) and T lymphocyte (CD3+ cell) dose, severe GVHD can be reduced whilst beneficial GVL effects can be preserved. We found that T cell depleted transplants using the Nexell/Baxter Isolex 300i system and subsequently, the Miltenyi CliniMACS® CD34+ system to obtain high CD34+ doses depleted of lymphocytes were safe to administer and associated with less severe acute GVHD and promising response rates and overall survival. Our previous trials have helped us to create the transplant environment (significant lymphodepletion and minimal post-transplant immunosuppression) that make for an ideal platform for adoptive cellular immunotherapy. Adoptive cell transfer is the passive transfer of immune cells, into a new recipient host with the goal of transferring the immunologic functionality and characteristics into the new host.

This protocol is designed to evaluate the safety and efficacy of the Miltenyi CliniMACS® CD 34 selection system in HLA-matched sibling allogeneic peripheral blood stem cell transplant. The manipulation of the graft is the primary research intervention, subject to IDE# 15632, and all other aspects of clinical management on this protocol are standard care. The target CD34+ dose range will be $>3 \times 10^6$/kg and the target CD3+ dose range will be 5×10^4/kg to 1×10^6/kg. Once we demonstrate adequacy of this platform for engraftment and absence of significant GVHD in ten consecutive recipients, we will seek IRB permission to proceed with planned adoptive cellular therapies.

The protocol will accrue up to 96 transplant recipients aged 10-80 with a hematological malignancy and their HLA-matched sibling donors, in whom allogeneic stem cell transplantation from an HLA-matched sibling would be routinely indicated. Diagnostic categories will include acute and chronic leukemia, myelodysplastic syndromes, lymphomas, multiple myeloma and myeloproliferative syndromes.

Subjects will receive a myeloablative conditioning regimen of cyclophosphamide (120 mg/kg total), fludarabine (125 mg/m^2 total) and total body irradiation (1200 cGy with lung shielding to 600 cGy), followed by an infusion of a stem cell product selected for CD34+ progenitors using the Miltenyi CliniMACS® system. Older subjects will receive a lower dose of irradiation (800 or 600 cGy based on age) to reduce the regimen intensity.

The overall objective is to assess the feasibility of using this system as a platform for cellular immunotherapy initiatives. The primary study endpoint will be overall survival at day +200. Stopping criteria for safety will monitor non-relapse mortality at day +200. Secondary endpoints will be standard transplant outcome variables such as non-hematologic toxicity, incidence and severity of acute and chronic GVHD and relapse of disease.

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SEE ALSO NIH BMT CONSORTIUM SUPPORTIVE CARE GUIDELINES
[http://intranet.cc.nih.gov/bmt/] NHLBI, IRB reviewed January, 2012.

1.0 OBJECTIVES

1.1 To determine the safety and efficacy of using the Miltenyi CliniMACS CD34 selection system for graft manipulation in HLA-matched sibling allogeneic stem cell transplantation.

1.2 To characterize the feasibility of using the Miltenyi CliniMACS CD34selection system as a platform for adoptive cellular immunotherapy in HLA-matched sibling stem cell transplantation.

2.0 BACKGROUND

2.1 Allogeneic Stem Cell Transplantation

Allogeneic stem cell transplantation has long been recognized as a curative treatment for a variety of hematologic malignant diseases (Armitage 1994). Over time this technique has improved mainly through significantly diminished non-relapse mortality. One notable advance has been the use of reduced-intensity transplants for toxicity reduction and the use of T cell depletion for graft versus host disease (GVHD) control. However, these transplants often fail to control the hematological malignancy (Georges and Storb 2003, Marmont*, et al* 1991, Platzbecker*, et al* 2004). Relapse of disease is now the main problem (Barrett and Battiwalla 2011). Data from the Center for International Bone Marrow Transplant Research (http://www.cibmtr.org/) on over 30,000 transplants for hematological malignancies performed worldwide over the last 30 years shows that relapse rates have remained unchanged (20% for early disease, 40% for intermediate disease, and 60% for advanced disease subjects). Disease-free survival follows the same trend (65% for early disease, 40% for intermediate disease, and 20% for advanced leukemia).

2.2 Complications of Allogeneic Stem Cell Transplantation

Death after stem cell transplantation can be categorized as relapse and non-relapse mortality (NRM). NRM includes GVHD, infection and organ toxicity.

2.2.1 Relapse

The curative potential of allogeneic stem cell transplantation is largely dictated by the stage of the disease (see 2.1 above) and disease free survival for advanced hematological malignancies has not changed significantly in recent years. To prevent relapse, subjects receive a chemo-radiotherapy conditioning regimen which is as intensive as they can tolerate. Based on previously reported experience for the treatment of elderly subjects with stem cell allografts we will use a reduced intensity conditioning regimen with fludarabine (Flu), cyclophosphamide (Cy) and reduced-dose total body irradiation (TBI) for subjects 55 years and older of age (Khoury et al. 2001, Weisser et al. 2004). The use of reduced-intensity conditioning for elderly subjects is a broadly accepted standard of care treatment and will not be part of the scientific questions addressed in this research protocol. In addition, there is a powerful immune response (graft-versus-leukemia, GVL effect) from the donor's T cells and natural killer cells. However because transplants also cause GVHD, it is necessary to give immunosuppression after the graft which reduces but does not eliminate GVHD, but decreases the GVL effect.

2.2.2 Graft Versus Host Disease

Graft-versus-host disease (GVHD) represents a major complication of allogeneic bone marrow transplantation (BMT) or peripheral blood stem cell transplantation (PBSCT), leading to significant

transplantation associated morbidity and mortality. Despite prophylaxis with cyclosporine (CSA) or methotrexate (MTX) or both, acute GVHD occurs in 30% to 50% of subjects receiving transplants from HLA-identical siblings. Long term survival in subjects developing severe acute GVHD has generally been less than 30% (Barrett 1993, Barrett 1997, Beatty*, et al* 1991, Storb*, et al* 1986, Weisdorf*, et al* 1990).

2.2.3 Infection

Following allogeneic stem cell transplantation, subjects are at increased risk of bacterial, fungal and viral infections. Pre-emptive antiviral agents, antibiotic fever regimens, and availability of effective new antifungal agents have greatly improved the management of infection after transplant. Infectious death now mainly occurs when infection complicates GVHD in the setting of intense immunosuppression.

2.2.4 Other complications

Some subjects die from major organ failure due to conditioning regimen toxicity and to the consequence of pre-transplant treatments for their malignancy. Depending on the underlying transplantation approach (e.g. conditioning regimen) the transplant can fail to engraft in up to 5% of cases.

2.3 Ex Vivo T Cell Depletion

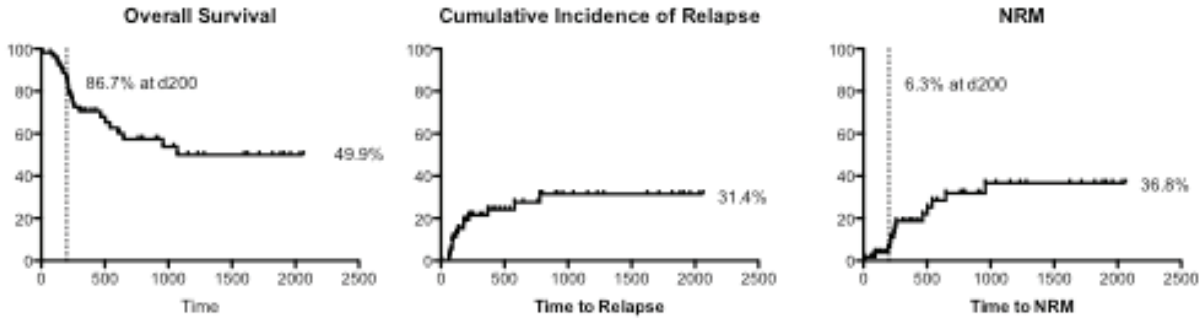
T cell depletion is a technique where lymphocytes are removed from allografts. T cell depleted transplantation can prevent GVHD but results in delayed immune recovery which increases the risk of viral infections (HSV, CMV or EBV), graft rejection as well as relapse of the malignant disease (Appelbaum 2001, Barrett 1993, Barrett 1997, Barrett and Malkovska 1996, Goldman*, et al* 1988, Marmont*, et al* 1991).

Prior Hematology Branch protocols utilized the Nexell/Baxter Isolex 300i system to obtain high CD34+ doses depleted of lymphocytes to a fixed CD3+ T cell dose. The use of the cell separator and the monoclonal antibodies was covered by IDE 8139 and the protocol series 99-H-0046, 02-H-0211, 03-H-0192, 03-H-0209 and 04-H-0112. Between 1997 and 2004, 138 subjects were treated on these protocols (Montero*, et al* 2006).They received a myeloablative T cell depleted peripheral blood stem cell transplant (PBSCT) from an HLA-identical sibling donor. The T-cell dose was adjusted to 0.2-1x10^5 CD3+ cells/kg. The CD34+ dose was 2.7–16 x10^6/kg. Subjects with less than grade II acute GVHD received 1 or 2 donor lymphocyte infusions (DLI) of 10^7 CD3+ cells/kg between days 45 and 100. Subjects were designated as standard (n=77) or high (n=61) relapse risk according to their pre-transplant characteristics. Overall survival (OS), relapse free survival, relapse and non-relapse mortality (NRM) were 58%, 46%, 40% and 20%, respectively, after a median follow up of 4 years. Fifty-three (39%) and 21(15%) subjects developed grade II-IV and III-IV acute GVHD. Forty-two (36%) had limited and 29 (25%) extensive chronic GVHD. In multivariate analysis, disease risk was an independent factor for OS and relapse, the day 30 lymphocyte count (LC30) for OS and NRM, and chronic GVHD for OS and relapse. In summary, these results show promising overall survival with low non-relapse mortality.

In 2006, we utilized the Miltenyi CliniMACS immunomagnetic selection system to select for CD34+ cells (IND 13058)achieving ~4-log CD3+ lymphocyte depletion on protocol # 06-H-0248(Battiwalla*, et al* 2011). At last analysis, the protocol continues to exceed its primary endpoint goal of day 200 overall survival >75%. Fifty-five subjects with hematologic

malignancies underwent allogeneic hematopoietic stem cell transplantation (HSCT) with a graft from their HLA-identical siblings. The median age was 43 years (range 13-68), 25/55 were males. Transplant indications were AML(27), ALL(14), acute biphenotypic leukemia(2), MDS/MPD(6), NHL/CLL(4), CMMoL(1) and CML(1). 47% were standard risk and 53% were at high risk for relapse. Subjects received myeloablative conditioning with cyclophosphamide (60 mg/kg/dose x 2), fludarabine (25 mg/m^2/dose x 5) and total body irradiation (12 Gy in 8 fractions, lungs shielded to 6Gy). Fifteen subjects, who were 55 years of age or older, received 4 Gy divided in 8 fractions without lung shielding. G-CSF mobilized peripheral blood grafts from the donor were CD34+ selected (Miltenyi CliniMACS), with infusion of a target CD34+ dose of $6x10^6$/kg and a fixed CD3+ dose of $5x10^4$/kg. Low-dose cyclosporine (100-200ng/mL) till day 21 was the only GVHD prophylaxis. Delayed lymphocyte add back ($5x10^6$ CD3+/kg) was given at day 90 if subjects had no significant GVHD. CD3+ and myeloid chimerism analyses were performed with early lymphocyte add-back in cases with falling chimerism. Day 200 overall survival (the primary study endpoint) was 86.7%. One subject, who was postpartum, failed to engraft and required a second transplant. Thirty seven out of 39 subjects achieved complete (>95%) donor myeloid chimerism by day14. The median times to complete donor CD3+ chimerism were day30 for 12 Gy subjects and >6 months for 4 Gy subjects (Mann Whitney test, p=0.004). The incidence of acute GVHD grade II, III and IV were 29%, 18% and 0%, respectively for all subjects and significantly lower for those who did not receive the routine DLI. The incidence of chronic limited or extensive GVHD was 34 and 11%, respectively. At a median follow up of 2.5 years, Kaplan-Meier estimates of relapse, NRM and OS were 31%, 37% and 50% respectively without any significant difference based on conditioning intensity.

Figure 1: Overall survival, relapse and NRM observed in subjects treated on protocol 06-H-0248



Outside the NIH, equally encouraging results with ex vivo T cell depletion using CD34+ depletion have just been reported from the multicenter Clinical Trials Network (CTN) protocol #0303 (Devine*, et al* 2011). A total of 44 subjects with AML in CR1 (n = 37) or CR2 (n = 7) with a median age of 48.5 years (range, 21-59 years) received myeloablative chemotherapy and fractionated total body irradiation (1375 cGy) followed by immunomagnetically selected CD34-enriched, T cell depleted allografts from HLA-identical siblings. No pharmacologic GVHD prophylaxis was given. All subjects engrafted. The incidence of acute GVHD grade II-IV was 22.7%, and the incidence of extensive chronic GVHD was 6.8% at 24 months. The relapse rate for subjects in CR1 was 17.4% at 36 months. With a median follow-up of 34 months, DFS for all subjects was 82% at 6 months, and DFS for subjects in CR1 was 72.8% at 12 months and 58% at 36 months.

In 2012, protocol# 06-H-0248 was replaced by a more complicated ex-vivo graft manipulation protocol 12-H-0028, which also utilized the Miltenyi CliniMACS platform and combined CD34+ progenitor selection with depletion of CD3+ (T-lymphocytes) responsible for acute GVHD as well

as depletion of CD19+ (B-lymphocytes) responsible for chronic GVHD. This method was designed to preserve NK cells and CD34+ cells, while achieving a comparable degree (~ 3.5 log) of CD3+ depletion. Preservation of NK cells in protocol 12-H-0028 was intended to provide the theoretical advantage of reducing viral infections and relapse without impacting GVHD. We found that target cell doses were attained, however there was an unexpected increase in early acute GVHD when a T cell addback of $50x10^4$CD3+/kg was given (Table 1). In contrast, in subsequent subjects with a T cell addback as low as 5 x 10^4 CD3+/kg, (equating with the CD3+ dose in the 06-H-0248 protocol), subjects experienced low donor chimerism including outright engraftment failures despite all subjects having received myeloablative conditioning.

5	58	MDS	YES	600	06/19/12	50	8.76	No	No	None	NA
6	53	AML	NO	1200	07/03/12	50	6.5	No	No	1	18
7	55	MDS	YES	600	08/14/12	5	4.54	Yes	Yes	None	NA
8	28	AML	NO	1200	08/28/12	5	7.1	No	No	3	17
9	33	CML	YES	1200	09/16/12	5	6.75	Yes	Yes	None	NA
10	38	CML	YES	1200	10/16/12	5	6.6	Yes	No	1	38
11	40	AML	NO	1200	10/23/12	5	6.15	No	No	None	NA

The reason for these outcomes on 12-H-0028 is unclear, but cannot be simply explained on the basis of CD3+ dose alone. More problematic is that the dynamic range of CD3+ dosing is unacceptably narrow (1-log), and not controlled by adjusting the CD3+ addback dose. In summary, while overall survival on 12/16/2012 was 100% for the cohort of 13 subjects transplanted, the unpredictability of outcome, characterized on the one hand by early significant acute GVHD and on the other hand by poor engraftment make 12-H-0028 unacceptable for continued development as a platform for adoptive cellular immunotherapy.

The current protocol will revert to positive CD34+ selection as used in the 06-H-0248 protocol with minor further improvements based on experience with this protocol and 12-H-0028.

2.4 SCIENTIFIC AND CLINICAL JUSTIFICATION

The focus of the NHLBI Hematology Branch allogeneic PBSCT program is to improve the treatment of hematologic and non-hematologic malignant diseases using transplant approaches that minimize GVHD, non-relapse mortality and increase the GVL effect.

Ex Vivo T Cell Depletion:

Our prior T cell depletion protocols achieved promising results for overall survival and significantly reduced non-relapse mortality (Battiwalla*, et al* 2011, Montero*, et al* 2006).The extramural published experience with CD34+ selection also shows reductions in GVHD without increase in relapse(Devine*, et al* 2011). The Isolex system employed for stem cell selection up to 2005was withdrawn from the market. The CliniMACSCD34 selection system preservesCD34+ cells (yield is reliably >50% but typically 75%), but induces a 4- log reduction in T lymphocytes (responsible for acute GVHD) along with a similar degree of reduction in B lymphocytes (contributes to chronic GVHD). We expect the final CD3+ counts at the end of the selection process to range between $5x10^4$/kg to $1x10^6$/kg, providing sufficient protection from GVHD on the one hand, and yet providing adequate T cells for safe engraftment on the other hand. The delayed T cell add-back at day 90 in protocol 06-H-0248 has not been particularly beneficial in the last protocol except for correcting poor donor CD3 chimerism, and will be no longer be given routinely. Low dose CSA will continue as the only immunosuppression at least until day +21, or until engraftment is stable. However, CSA cessation will provide an immunosuppression-free environment for the T cells to expand sufficiently and develop strong GVL effects as required for relapse-control. We will select

subjects with hematological malignancies for whom allogeneic stem cell transplantation from a fully matched donor is considered a standard, uncontroversial treatment. We regularly review the place of stem cell allotransplantation in the treatment of hematological malignancies, particularly chronic myelogenous leukemia (CML), where tyrosine kinase inhibitors such as imatinib and newer agents have made first line transplantation obsolete. Indications for transplantation are regularly reviewed by the DSMB.

3.0 DISTINCTIVE FEATURES OF THIS PROTOCOL

Distinctive features of this protocol are shown in context of serial protocol development in **Table 2**.

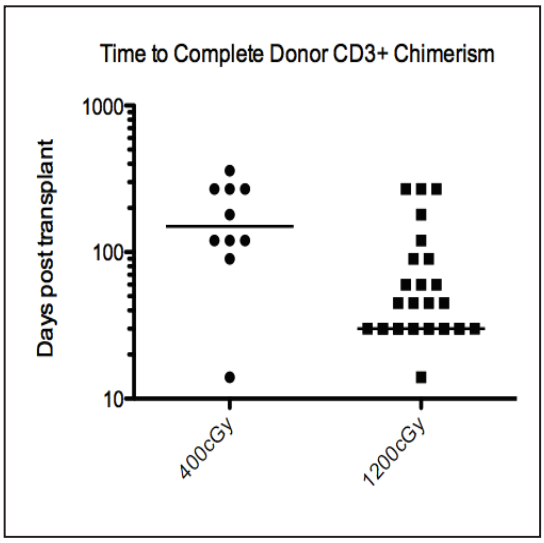
Table 2: SEQUENTIAL PROTOCOL CHANGE SUMMARY TABLE

Features	***06-H-0248***	***12-H-0028***	***This Protocol (prior to 12/2015)***	***This Protocol (after 12-2015)***
Graft manipulation	CD34+ selection	CD 3/19 depletion and CD34+ selection	CD34+ selection	CD34+ selection
Dose range for CD3+ addback at day 0	1 to 5 x 10e4/kg	5 to 100 x 10e4/kg	5 to 100 x 10e4/kg	0 to 100 x 10e4/kg
Conditioning TBI *<55 years*	1200 cGy	1200 cGy	1200 cGy	1200 cGy
≥55 years	400 cGy	600 cGy	600 cGy	*≥55 to <65 yrs =1200 cGy; ≥65 to <75 yrs =800 cGy; ≥75 to 80 yrs =600 cGy*
Routine DLI	at day 90	none	none	none
Platform for adoptive cellular therapy	No	Proposed but failed	***Proposed***	***Multivirus T lymphocytes***

Generation of T cell depleted stem cell product: We will continue to utilize the Miltenyi CliniMACS system but will revert to using CD34+ selection alone to deplete the donor stem cell graft of lymphocytes. The Miltenyi CliniMACS is a clinical-scale immunomagnetic system widely used in Europe and is available in the USA under an IND/IDE. This system uses CD34antibody-coated magnetic bead reagent manufactured for clinical use. The National Institutes of Health (NIH) Department of Transfusion Medicine (DTM) Cell Processing Section has extensive experience in generating CD34+ enriched stem cell products.

Intensity of the TBI conditioning for older subjects (≥55 years): In this protocol, we will continue to include older subjects. Based on previously reported experience for the treatment of the elderly we will use reduced intensity conditioning with Flu/Cy and reduced dose TBI for these subjects (Khoury et al. 2001, Weisser et al. 2004). However, in the 06-H-0248 protocol, 400 cGy TBI resulted in sluggish conversion to full donor CD3+ chimerism compared to 1200cGy (median 180 days versus 30 days, $p < 0.001$). This has often required premature DLIs to rescue failing grafts.

Figure 2: Protocol 06-H-0248 – Slow donor CD3+ engraftment for older recipients conditioned with 400cGy TBI



We will therefore increase the intensity of TBI to 600cGy for the reduced-dose cohort in this protocol. This increase to 600 cGy was well tolerated in subjects above 55 years in the 12-H-0028 protocol and will continue.

The TBI dose will be further increased for some of the older subjects by protocol amendment since 12/2015 in a graded fashion. [Age ≥55 to <65 yrs =1200 cGy(with lung shielding to 600 cGy); ≥65 to <75 yrs = 800 cGy (with lung shielding to 600 cGy); ≥75 to 80 yrs =600 cGy] The rationale is to reduce the risk of relapse (which is usually lethal), currently at 58% in subjects over age 55. TBI at 600cGy has been extremely well tolerated in these subjects over age 55 and the TRM rate is actually 0% at the time of this amendment, which makes room for increasing TBI dose. The justification is based upon the relatively lower toxicity of our TBI approach, measures taken to reduce TBI toxicity, existing practice at other centers and evolving evidence to maximize conditioning intensity when possible.

Avoidance of Routine Donor Lymphocyte Infusion (DLI): Before PBSCT, the donor will undergo leukapheresis in order to collect lymphocytes for possible future transfusion to the recipient. The leukapheresis collection will be divided and stored as 2 or more products in DTM. In our previous protocols, we had gradually postponed a DLI till day 90. The extramural experience with T lymphocyte depletion has avoided delayed DLI entirely (Devine*, et al* 2011). Additionally, both acute and chronic GVHD were increased in frequency and grade in 06-H-0248 transplant recipients who received DLIs. The requirement for DLI has been dropped since protocol 12-H-0028. Similarly, in this protocol, donor lymphocytes will be collected and stored in advance but only administered for standard clinical indications: for relapse, falling or delayed chimerism.

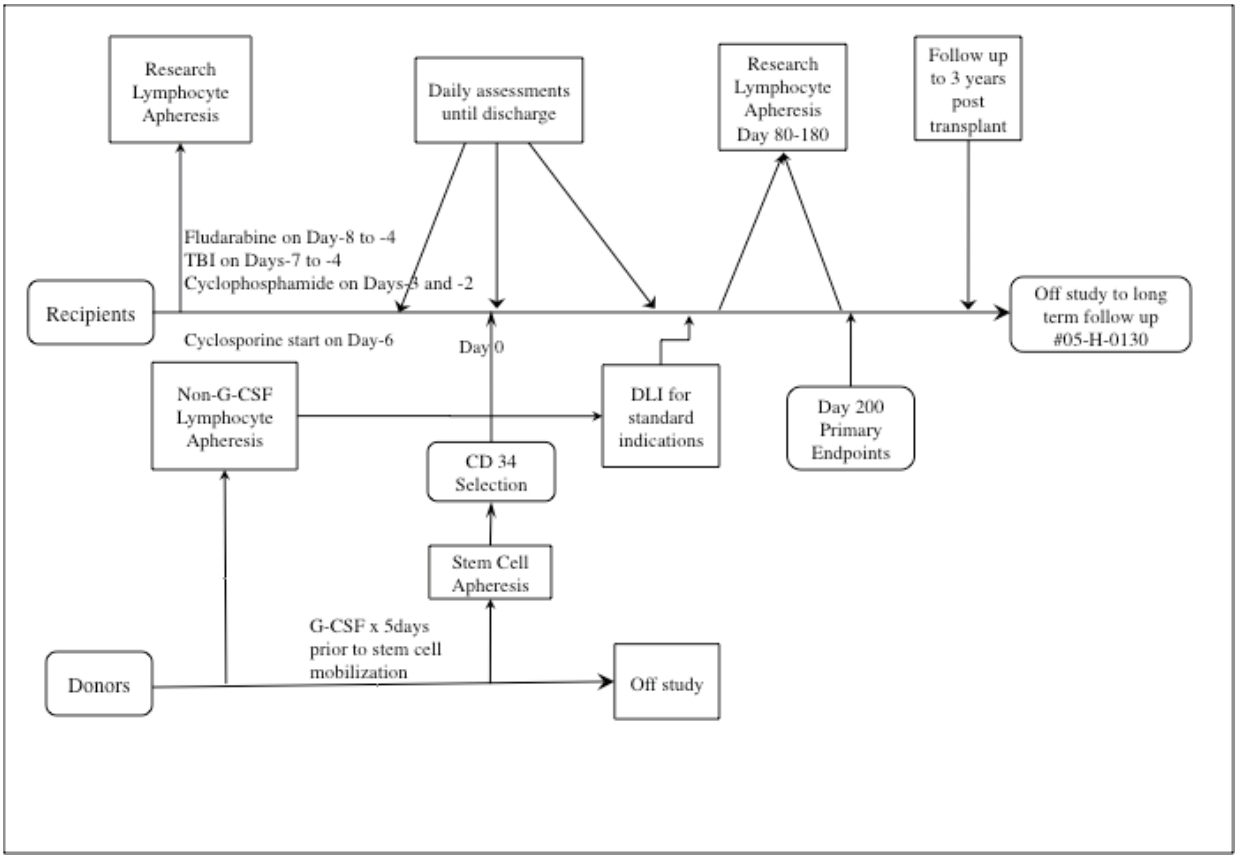
Platform for adoptive immunotherapy strategies: Our goal for advancing allogeneic HSCT strategies is to develop a T cell depletion platform for adoptive cellular immunotherapy to minimize GVHD and optimize antiviral and antimalignancy immune responses. In sequential clinical trials at NIH we have used ex-vivo T cell depletion to develop an HSCT with minimal graft versus host disease (GVHD) prophylaxis comprising low dose cyclosporine alone till day +21. Tcell depletion creates the lymphodepleted environment for preferential homeostatic expansion of adoptively infused cells, while minimization of post-transplant immunosuppression promotes expansion of the adoptively transfused cells. The adoptive transfer concept will be explored in this protocol, once 10 consecutive subjects have enrolled and demonstrated satisfactory outcomes (full engraftment in at least 9 subjects and acute GVHD of - grade II-IV<40%), Section 11.5. This criterion has been met

as of May, 2014 and formal IRB approval will permit utilization of this protocol as a transplant platform for additional immunotherapy protocols: adoptive cellular immunotherapy against malignancy and viral infections, cancer vaccines, donor IL-2 derived cellular products, bone marrow stromal cell delivery and donor vaccinated cellular products.

4.0 STUDY DESIGN

This is a phase I-II clinical trial designed to evaluate the safety and efficacy of the CliniMACS CD34 selection system as a T cell depletion technique and, as a primary endpoint, to determine overall survival at day +200 after transplantation. Recipients will receive a myeloablative conditioning regimen of cyclophosphamide, fludarabine and total body irradiation, followed by an infusion of a stem cell product prepared using the Miltenyi CliniMACS system.

Figure 3: Schema



5.0 ELIGIBILITY ASSESSMENT

Subjects in remission as well as subjects with primary induction failure or refractory disease will be considered for inclusion. At the discretion of the Principal Investigator (PI), subjects may continue standard of care treatment options to control their baseline disease burden up to the start of the protocol as detailed in section 7.1.

5.1 Inclusion Criteria-Recipient

5.1.1 Ages 10-80 years inclusive

5.1.2 Any one of the following hematologic conditions, confirmed by pathology, meeting a standard indication for allogeneic stem cell transplant:

5.1.2.1 Chronic myelogenous leukemia (CML): Subjects under the age of 21 in chronic phase OR Subjects ages 10-80 in chronic phase who have failed or are intolerant to treatment with second generation tyrosine kinase inhibitors OR Subjects ages 10-80 in accelerated phase or blast transformation**.** ***OR***

5.1.2.2 Acute lymphoblastic leukemia (ALL): any of these categories: Adult ALL including standard risk; Pediatric ALL in first remission with high-risk features (presenting leukocyte count >100,000/cu mm, karyotypes t (9; 22), t4, t19, t11, biphenotypic leukemia). All second or subsequent remissions, primary induction failure, partially responding or untreated relapse. ***OR***

5.1.2.3 Acute myelogenous leukemia (AML): AML in first remission - *except* AML with good risk karyotypes: AML M3 (t15; 17), AML M4Eo (inv 16), c-kit unmutated AML t (8; 21). All AML in second or subsequent remission, primary induction failure and resistant relapse. ***OR***

5.1.2.4 Myelodysplastic syndromes(MDS): any of these categories - refractory anemia with transfusion dependence, refractory anemia with ANC<500/μL, refractory anemia with excess of blasts, transformation to acute leukemia, chronic myelomonocytic leukemia, atypical MDS/myeloproliferative syndromes.***OR***

5.1.2.5 Myeloproliferative disorders including atypical (Ph-negative) chronic myeloid and neutrophilic leukemias, progressing myelofibrosis, and polycythemia vera, essential thrombocythemia either in transformation to acute leukemia or with progressive transfusion requirements or pancytopenia. ***OR***

5.1.2.6 Chronic lymphocytic leukemia refractory to fludarabine treatment and with bulky progressive disease or with thrombocytopenia (≤100,000 /□μl) or anemia (≤10g/dl) not due to recent chemotherapy. ***OR***

5.1.2.7 Non-Hodgkin's lymphoma including Mantle cell lymphoma relapsing or refractory to standard of care treatments. ***OR***

5.1.2.8 Multiple myeloma, Waldenstroms macroglobulinemia, unresponsive or relapsed following standard of care treatments. ***OR***

5.1.2.9 Hodgkin's Lymphoma relapsing following an autologous transplant. ***OR***

5.1.2.10 Other rare hematologic malignancies for which hematopoietic stem cell transplantation has been performed and offers a durable remission or as the only option for potential for cure.

- Chemotherapy-resistant multisystem Langerhans cell histiocytosis (MSLCH) especially involving organs like the bone marrow, liver, spleen, and lungs
- Aggressive systemic mastocytosis, and mast cell leukemia (MCL) in first CR (CR1)
- Hypereosinophilic syndrome who have failed imatinib therapy or FIP1L1-PDGFRa-negative patients who develop end-organ dysfunction
- Adult T-cell leukemia/lymphoma at first diagnosis
- Refractory or disseminated nasal-type extranodal NK/T-lymphoma or aggressive Natural killer cell leukemia/lymphoma
- Mycosis fungoides and Sézary syndrome after failure of two or three initial therapies
- Primary or relapsed refractory Angioimmunoblastic T-cell lymphoma at first diagnosis
- Hepatosplenic T-cell lymphoma (gamma/delta T-cell lymphoma) at first diagnosis

- T-cell prolymphocytic leukemia at first diagnosis
- Subcutaneous panniculitic T-cell lymphoma at first diagnosis
- Hematodermic neoplasm (blastic natural killer cell lymphoma or Blasticplasmacytoid dendritic cell neoplasm) at first diagnosis

5.1.3 HLA identical (6/6) related donor.

5.1.4 For adults: ability to comprehend the investigational nature of the study and provide informed consent. For minors: written informed consent from one parent or guardian. Informed oral assent from minors: the process will be explained to the minor on a level of complexity appropriate for their age and ability to comprehend.

5.2 Exclusion Criteria – Recipient (any of the following)

5.2.1 Major anticipated illness or organ failure incompatible with survival from transplant
5.2.2 Severe psychiatric illness or mental deficiency sufficiently severe as to make compliance with the transplant treatment unlikely and making informed consent impossible.
5.2.3 Positive pregnancy test for women of childbearing age
5.2.4 DLCO adjusted for Hb and ventilation< 50% predicted
5.2.5 Left ventricular ejection fraction < 40% (evaluated by ECHO) or < 30% (evaluated by MUGA)
5.2.6 AST/SGOT > 10 x ULN
5.2.7 Total bilirubin > 5 x ULN
5.2.8 Estimated GFR < 15 mL/min
5.2.9 Recipients who have active infections with HIV or active hepatitis C (HCV)

5.3 Inclusion criteria- Donor

5.3.1 Related donor, HLA identical (6/6) with recipient
5.3.2 Weight $\geq$ 18 kg
5.3.3 Age $\geq$ 2 or $\leq$80 years old
5.3.4 For adults: ability to comprehend the investigational nature of the study and provide informed consent. For minors: written informed consent from one parent or guardian and informed assent: The process will be explained to the minor on a level of complexity appropriate for their age and ability to comprehend.

5.4 Exclusion Criteria - Donor (any of the following)

5.4.1 Pregnant or breast-feeding. Lactating donors are permitted provided breast milk is discarded during the days filgrastim (G-CSF) is given
5.4.2 Unfit to receive G-CSF and undergo apheresis (abnormal blood counts, history of stroke, uncontrolled hypertension)
5.4.3 Sickling hemoglobinopathy including HbSS, HbSC
5.4.4 Donors who are positive for HIV, active hepatitis B (HBV), hepatitis C (HCV) or human T-cell lymphotropic virus (HTLV-I/II)
5.4.5 Severe psychiatric illness or mental deficiency sufficiently severe as to make compliance with the donation process unlikely, and making informed consent impossible.

6.0 CLINICAL EVALUATION OF THE TRANSPLANT RECIPIENT

Samples will be ordered and tracked through the CRIS order sets. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record.

6.1 Pre-Study Evaluation (within 3 months of transplant)

Transplant recipients may also be screened on the Hematology Branch screening protocol (97-H-0041 or its replacement) or in the patient's NIH medical record.

- History and physical exam, waist circumference
- HLA-A, B &DR-B1 typing, KIR typing (no time frame)
- Bone marrow aspirate and biopsy. Chromosome analysis, if clinically indicated. Minimal residual disease markers and any other disease-appropriate staging (such as a CT-PET scan)
- BCR-ABL (CML, and Ph+ ALL only) or other disease-specific molecular test
- Serum HbsAg, anti-HBs, anti-HBc, anti-HCV, HAV, anti-HIV, anti-HTLV-1, anti CMV, anti-EBV, anti-Toxoplasma, RPR, PPD
- PCR: blood for CMV/EBV, Toxoplasma, HHV6, BK virus, adenovirus and urine for adeno/BK virus
- Red cell ABO, Rh, antibody screen,
- HLA alloimmunization screen (where clinically indicated)
- Coagulation Screen, Fibrinogen
- CBC with differential
- Reticulocyte count
- Acute care (Na, K, Cl, CO_2, Creatinine, Glucose, and Urea Nitrogen) panel
- Hepatic (Alk Phosphatase, ALT, AST, Total Bilirubin, and Direct Bilirubin) panel
- Mineral (Phosphorus, Magnesium, Albumin, and Calcium) panel
- Lipid panel
- LDH
- CK
- Uric acid
- Ferritin, Iron, Transferrin
- Urinalysis (UA)
- Quantitative immunoglobulins
- Lymphocyte Immunophenotyping (CD4, CD8, B, NK)
- Endothelial damage markers (vWF panel/FactorVIII activity, thrombomodulin, P-Selectin, Plasminogen Activation Inhibitor-Type 1, Pro BNP, haptoglobin, endothelin 1)
- Vitamin D levels (1,25 Dihydroxy and 25 Hydroxy)
- Pre albumin
- C-reactive protein
- Pregnancy test (serum or urine)
- STR profile (chimerism)
- Thyroid function, adrenal function, gonadal function, cortisol
- Growth hormone, growth chart, bone age (prepubertal children)
- Chest radiograph, pulmonary function tests including Vital capacity, FEV-1, DLCO
- Sinus CT scan and other body CT scans as medically required
- Cardiac function: EKG, ECHO, and/or MUGA scan
- Nutritional assessment, as needed
- Dental evaluation, as needed
- Social work consult
- Ophthalmology consultation as needed
- Gynecology consultation as needed

- The possibilities and limitations of semen storage for males and in vitro fertilization and storage of zygotes for females will be discussed with subjects as appropriate
- Durable power of attorney form completed
- Semen analysis (optional)
- Bone Mineral Density (optional)
- Health Related Quality of Life (PROMIS 29-Profile v1.0, PROMIS v1.0-Applied Cognition-General Concerns-Short Form, FACT-BMT, Sexual Functioning Questionnaire (SFQ) [Screening questions 1-3 and subscales: Medical Impact, Satisfaction and Problems, gender specific versions]). HRQOL is optional for the subject.
- All subjects age 50 or greater
    - Cardiac Stress test (stress nuclear perfusion imaging or treadmill stress test)
    - Cardiac consultation

6.2 In-Patient Monitoring (Day -8 to discharge - approximately day 30)

As the subjects' condition stabilizes and they approach hospital discharge this monitoring frequency may change as clinically indicated. These are only guidelines, the nature of clinical care and exact schedule of laboratory and questionnaire testing for these complex transplant subjects will depend on the clinical status and PI discretion.

- CBC with differential (daily)
- Acute care panel (daily)
- Hepatic panel (daily)
- Mineral panel (daily)
- Protein, Total (twice weekly)
- LDH (daily)
- CK (twice weekly)
- Uric Acid (twice weekly)
- Temperature, pulse, blood pressure, respiratory rate, weight, (daily)
- Reticulocytes (twice weekly)
- Prealbumin (twice weekly)
- CMV/EBV, Toxoplasma, HHV6, BK, adenoviral surveillance in blood and adeno/BK in urine (weekly when counts allow)
- C-reactive protein (weekly)
- Urine for viral cytopathic change (weekly)
- Lymphocyte Immunophenotyping (CD4, CD8, B, NK) (weekly)
- Coagulation screen (if clinically indicated)
- BCR-ABL (day 30 and as clinically indicated, CML and Ph+ ALL patient only)
- Stool for *C. difficile* (when clinically indicated)
- Bone marrow aspirate or peripheral blood to quantitate engraftment in subjects without evidence of hematologic recovery (as needed)
- Drug levels where appropriate (e.g., gentamicin, vancomycin, cyclosporine, voriconazole) (frequency variable)

6.3 Follow Up: After Initial Inpatient Hospitalization to Referral Back to Home Physician (typically 3-4 months)

Weekly (+/- 3 days): these are only guidelines, the nature of clinical care and exact schedule of laboratory testing for these complex transplant subjects will depend on the clinical status and PI discretion.

- CBC with differential
- Acute care panel
- Hepatic panel
- Mineral panel
- Protein, Total
- LDH
- CK
- Uric Acid
- Reticulocyte count
- Temperature, pulse, blood pressure, respiratory rate, weight,
- C-reactive protein
- Coagulation screen (if clinically indicated)
- Serum CMV/EBV, BK, adenoviral surveillance & Urine BK, adenoviral surveillance.
- Urine for viral cytopathic change (weekly)
- Lymphocyte Immunophenotyping (CD4, CD8, B, NK) (weekly)
- Vitamin D levels (1,25 Dihydroxy and 25 Hydroxy) (monthly)
- Drug levels as appropriate (i.e. cyclosporine)

Frequency as indicated: these are only guidelines, the nature of clinical care and exact schedule of laboratory testing for these complex transplant subjects will depend on the clinical status and PI discretion.

- BCR-ABL (day 60, 90 and +120 or as clinically indicated +/- 7 days, CML and Ph+ ALL subjects only)
- Chimerism profile (on days 14, 21, 28, 42, 60, 90 and as clinically indicated +/- 7 days)

6.4 Follow up after returning home

Subjects and/or their referring physician will remain in contact with the NIH and encouraged to send the results of laboratory testing at least every 3 months (+/- 2 weeks) for 200 days. At 3 years, subjects will be offered enrolment in the long-term follow-up protocol 05-H-0130 and communication with the patient and the referring physician is continued.

7.0 TREATMENT PLAN

7.1 Pre Transplant Treatment of Hematological Malignancy

Following enrollment on the Branch Standard of Care protocol (94-H-0010 or its replacement), subjects may receive any clinically indicated pre-transplant antineoplastic therapy according to standard indications to control their primary disease and increase the chance of successful transplantation. The PI will decide on the details of the treatment necessary in the individual case.

7.2 Apheresis of Transplant Recipient for Lymphocytes

With Amendment Q, Sept 2017, apheresis will not be performed on transplant recipients.
One 1 x 10^{10} cell apheresis of the recipient will be collected between day 80-180 post-transplant to study post-transplant immune function.

Research apheresis may be substituted by a 100 ml blood sample if apheresis is not feasible in terms of timing, recipient availability, scheduling constraints or the clinical condition of the recipient (blood counts and performance) at the discretion of the PI.

7.3 Central Venous Line Placement (See NIH BMT Consortium Supportive Care Guidelines**)**

7.4 Infection Prophylaxis and Treatment (See NIH BMT Consortium Supportive Care Guidelines**)**
Voriconazole or posaconazole will be the preferred antifungal prophylaxis, to begin on day +1.

7.5 Fever Regimen (See NIH BMT Consortium Supportive Care Guidelines**)**

7.6 Preparative Regimen

- **Fludarabine (Flu):** 25 mg/m^2/dose IV infusion once daily over approximately 30 minutes (e.g. 20 to 60 minutes) on Days -8, -7, -6, -5, and -4 (total dose of 125 mg/m^2). Dose adjustment for reduced creatinine clearance is required per the product label.

- **Total body irradiation (TBI)**:

  Energy
  All recipients should be treated with a linear accelerator using energies higher than 4MV.

  Timing
  It is anticipated that TBI will be delivered on days -7, -6, -5, -4

  Technique
  TBI will be delivered with lateral fields using extended SAD values of 600cm. For subjects receiving 1200 cGy, partial transmission lung blocks will be used combined with AP/PA Mediastinal Boost fields using full blocks in the lung area. Tissue compensators will be used as appropriate for all recipients. Gonadal shielding will not be used.

  Dose/Fractionation
  Recipients less than 65 years of age will be treated with TBI to a total dose of 1200 cGy delivered in 150 cGy fractions twice daily, at least 6 hours apart (eight total treatments in four days). The mediastinal boost fields will be delivered at a dose of 75cGy per fraction for a total dose of 600cGy, also at least six hours apart. The median lung dose goal is 600 cGy from the total treatment.

  Recipients 65 years to <75 years will receive TBI to a total dose of 800 cGy delivered in 100 cGy fractions bid, at least 6 hours apart (eight total treatments in four days). The mediastinal boost fields will be delivered at a dose of 50 cGy per fraction for a total dose of 400cGy. The median lung dose goal is 600 cGy from the total treatment.

  Recipients 75 years and older will receive TBI to a total dose of 600 cGy delivered in 75 cGy fractions bid, at least 6 hours apart (eight total treatments in four days). No mediastinal boost field will be used.

  Dose Modifications
  Occasionally, the total dose/technique of TBI may require modifications due to patient factors (unexpected or serious (grade 4-5) adverse events, serious medical illnesses not conducive to stable patient transfer, patient refusal, etc.) or treatment factors (linear accelerator machine offline, etc.) Modifications to the radiation treatment will be at the discretion of the treating radiation oncologist and will be discussed with the PI.

- **Cyclophosphamide (Cy)** 60 mg/kg/dose IV infusion administered over approximately 1 to 2 hours once daily for 2 doses on Days -3 and -2 (total dose of 120 mg/kg). Infusion rate may be extended if necessary for infusion associated toxicities. Mesna and intravenous hydration will be given concurrently with the cyclophosphamide doses as based on current NIH BMT Consortium Supportive Care Guidelines.

7.7 Stem Cell Transplantation Day 0

The target and acceptable range of cell doses (given in terms of recipient body weight) are:

CD34+ cells

Target dose: 6 x 10^6/kg recipient weight

Acceptable range: maximum- None; minimum- 3 x 10^6/ kg

CD3+ cells (T cell add back)

Target dose: Based upon our experience with previous protocols 06-H-0248 and 12-H-0028, for relatively chemo-naïve subjects (e.g. MDS, CML), and for those who receive 600cGy TBI, the target dose will be 5 x 10^5CD3+/kg. All other subjects will receive 5 x 10^4 CD3+/kg. Target doses may be further individualized based upon engraftment kinetics as the protocol progresses. As of amendment dated July 1, 2016, subjects receiving adoptive cell therapy such as multiviral T lymphocytes between days 0 to 7 post graft will not require the CD3+ addback.

Acceptable range: maximum 1 x 10e6 CD3+/kg; minimum 0 CD3+/ kg

The source of CD3+ cells will be an aliquot of the G-CSF mobilized apheresis product.

If the final CD3+ dose is greater than 1 x 10^6/kg this is designated a technical failure of the selection process. Recipients will be transplanted with this final product (and additional methotrexate GVHD prophylaxis- Section 8.5) but their outcome will be analyzed separately.

The ex vivo processing and required product safety testing is detailed in section 8.5.

7.8 GVHD prophylaxis

Cyclosporine (CSA) will be initiated at a dose of 1.5 mg/kg IV every twelve hours day –6 to day +21 to reduce the risk of graft failure. (Change to equivalent oral dose when able to tolerate with dose adjusted to achieve levels 100-200 ng/ml). The initial cyclosporine dose (1.5 mg/kg IV every 12 hours) will be based on the recipient's actual body weight unless the recipient is obese (body mass index > 35); in which case the cyclosporine dose will be based on an adjusted / practical body weight (mid-point between ideal body weight and actual body weight; see NIH BMT Consortium Supportive Care Guidelines). Cyclosporine may continue beyond day 21 if there is risk of incomplete donor CD3+ engraftment. Cyclosporine may be substituted at the discretion of the PI based upon tolerance or potential toxicity.

Subjects who have a technical failure of graft processing and receive a CD3+ dose >1 x 10^6/kg, will receive additional methotrexate GVHD prophylaxis at a dose of 5mg/m2 which will be given by IV infusion over 15 minutes post-transplant on days 1,3 and 6. Methotrexate dose adjustments for renal or hepatic dysfunction will be based on NIH BMT Consortium Supportive Care Guidelines.

7.9 Transfusion Support: Filtered and irradiated blood products as needed. HLA-alloimmunized recipients will be identified by history and confirmed by lab testing. Premedications will be given as clinically required.

7.10 Nutrition: Parenteral nutrition will be instituted as clinically necessary.

7.11 Hospital Discharge: The recipient will be in the hospital for about 3-6 weeks and will be discharged when clinically indicated to follow up as an outpatient.

7.12 Donor lymphocyte Infusion (DLI)

Donor lymphocyte infusions will not be routinely administered at a fixed time point in this protocol. At the discretion of attending physician up to 5x 10^8 CD3+ cells/kg DLI can be given at any time post-transplant for standard clinical indications: in overwhelming viral infections, falling donor chimerism, delayed chimerism or in subjects who appear to be at risk of disease relapse, or are in frank relapse. The ex vivo processing and required product safety testing is detailed in section 8.

7.13 Antiviral Cellular Therapy

With amendment version 6-5-14, subjects may qualify to participate in a concurrent protocol for testing the safety and efficacy of transfer of multiviral specific T lymphocytes in reducing common post-transplant viral infections.

7.14 Misc. Supportive Care

Palifermin may be selectively used for subjects at high risk of mucositis (such as those receiving a cranial irradiation boost) and ursodiol will be routinely used as prophylaxis against hepatic complications. Other aspects of supportive care will follow NIH BMT Consortium Supportive Care Guidelines.

8.0 DONOR EVALUATION, STEM CELL COLLECTION AND PROCESSING PLAN

8.1 Pre-Study Consult and Evaluation

Donors may also be screened on the Hematology Branch screening protocol (97-H-0041 or its replacement).

- HLA, -A -B –C -DR typing, KIR typing
- Confirm HLA compatibility with patient (6/6 HLA match).
- Rule out sickling hemoglobinopathies including HbSS and HbSC by history and peripheral blood smear. Donors with HbAS are acceptable
- History and physical examination
- Chest X-ray in donors with underlying pulmonary disease or history of smoking
- HbsAg, anti-HBc, anti-HCV, anti-HIV, anti-HTLV, anti-CMV, RPR, anti- T. cruzi, West Nile virus NAT (nucleic acid test) and human transmissible bovine spongiform encephalopathy (BSE is done by questionnaire of associated risk factors)
- Red blood cell ABO group, Rh type, antibody screen
- CBC with differential, coagulation screen, Acute Care, Mineral, Hepatic, and Other (Total Protein, CK, Uric Acid, and LD panel), pregnancy test (females of child bearing potential)
- Profile STR (chimerism)

- Fit to donate: Orientation - visit to Department of Transfusion Medicine - inspection of veins to determine the need for a central line for apheresis. It is estimated that about 50% of the donors will require apheresis catheter placement to successfully complete apheresis.
- Counseling on filgrastim (G-CSF) mobilization and donation of leukocytes

8.2 Pre-consent Evaluation and Concurrent Care of Minor Donors (donors less than age 18 only)

For donors less than age 18, a social worker and/or mental health specialist (psychologist or psychiatrist) will meet with the minor prior to the assent process to confirm willingness to participate. For donors less than age 18, a separate pediatric provider who is charged solely to consider the health and welfare of the minor donor will be provided. This practitioner will serve as the donor's health care provider and advocate during the minor's participation on the clinical trial.

8.3 Donor Lymphocyte Collection – Pre-transplant

One 10-15 liter apheresis will be collected prior to or 2 or more days after filgrastim (G-CSF) mobilization and stem cell collection, to obtain 1 x 10^{10} lymphocytes for cryopreservation of DLI (at least one aliquot of 5 x 10^6/kg, additional aliquots for clinical use, and an aliquot for in vitro laboratory research studies [section 10.0]). If a central catheter is necessary, please refer to NIH BMT Consortium Supportive Care Guidelines.

8.4 Donor Mobilization with G-CSF

After medical evaluation and clearance for suitability as an allogeneic donor by the BMT service in consultation with DTM, the donor will undergo mobilization with filgrastim (G-CSF) as an outpatient. Filgrastim (G-CSF) will be administered based on body weight (see below) for up to 6 days, subcutaneously. Filgrastim (G-CSF) will be administered according to a vial-based algorithm to reduce wastage, improve donor compliance, and optimize CD34 yields. The doses for days 1-4 may be given at any time of day, but the doses for days 5 and 6 must be given very early in the morning, at least one hour prior to starting apheresis. Predictable side effects of filgrastim (G-CSF), including headache, bone pain, and myalgia, will be treated with acetaminophen or ibuprofen or oxycodone. Prophylactic treatment of these side effects with the same medications may be elected. Other side effects will be evaluated and treated accordingly.

Donor Wt	Total daily filgrastim (G-CSF) Dose (range)
19-30 kg	300 mcg (10.0 to 15.8 mcg/kg)
31-37 kg	480 mcg (10.0 to 13.0 mcg/kg)
38 - 48 kg	600 mcg (12.5 to 15.8 mcg/kg)
49 - 56 kg	780 mcg (13.9 to 15.9 mcg/kg)
57 - 60 kg	900 mcg (15.0 to 15.8 mcg/kg)
61 - 67 kg	960 mcg (14.3 to 15.7 mcg/kg)
68 - 108 kg	1080 mcg (10.0 to 15.9 mcg/kg)
> 109 kg	1200 mcg (11.0 or less)

8.5 Peripheral Blood Stem Cell Collection &Ex Vivo Processing

PBSC apheresis will be done on days 5 and 6 of filgrastim (G-CSF), i.e., after the 5th and 6th doses of filgrastim (G-CSF) (which are given in the early morning). All apheresis procedures will be done using a 2-armed approach or by temporary central venous catheter using the Cobe Spectra or other blood separator in use in DTM. If a central catheter is necessary, please refer to NIH BMT Consortium Supportive Care Guidelines.

Anticoagulation will be accomplished with ACD-A. If the donor is small or intolerant to ACD-A, and the adverse citrate effects cannot be controlled by usual means (slowing flow rate, oral or IV calcium), consideration will be given to using heparin anticoagulation.

Donors will receive divalent cation prophylaxis to prevent citrate toxicity during apheresis, in accordance with standard DTM policies. The volume processed per apheresis procedure will be determined by DTM medical staff on the day of apheresis, based on peak CD34 cell mobilization response to GCSF and the CD34 cell dose needed, based on kilogram weight of recipient. This will range from 15 to 30 liters processed per day for up to 2 days, not to exceed a total of 60 liters over 2 days. In donors weighing less than 40 kg, a maximum of 8 blood volumes will be processed per day, for up to 2-3 days.

The second day apheresis collection will not be required if the CD34+ dose is 4 $x10^6$/kg or more after the first day of collection, processing and selection.

The aim is to provide a CD34+ selected graft with a minimum of 3x 10^6/kg and a maximum of 10 x 10^6/kg CD34+ cells within the constraint of a maximum CD3+ cell dose of 1 x 10^6kg.

If the minimum CD34+ cell dose is not achieved after the first apheresis and if it is predicted, based on the circulating CD34+ cell count prior to the first apheresis and the CD34+ yield in the apheresis component, that the minimum cell dose will not be achievable with a second consecutive apheresis, then plerixafor may be added to a sixth dose of filgrastim prior to the second procedure. The dose of plerixafor is 0.24 mg/kg SC given at 10 PM the evening prior to the second apheresis. The sixth dose of filgrastim should be given in the early AM, at least one hour prior to the start of the second apheresis procedure.

If the minimum CD34+ cell dose has not been achieved after one cycle of mobilization, a 2nd mobilization with up to two full apheresis collections to achieve the minimum post-selection CD34+ cell dose may be performed after an interval of at least 14 days.

In the event insufficient number of CD34+ cells are mobilized to allow for efficient selection by the Miltenyi system during the first round of apheresis, subsequent courses of mobilization will only be processed through the Miltenyi system if the minimum target of total CD34+ cell dose (3 x 10^6/kg) is predicted to be obtained following Miltenyi selection. If the post Miltenyi processing total CD34+ cell dose is predicted to be less than the minimum total target CD34+ cell dose, the CD34 positively selected cells from the first apheresis procedure will be combined with the CD34+ *unselected* cells collected following subsequent mobilization procedures so that the minimum target stem cell dose is provided.

Because the unselected CD34+ fraction (containing the required additional stem cell number) will retain all lymphocytes, the graft will be considered a T-replete stem cell graft with associated increased risk of GVHD. This technical failure of the mobilization process will be reported to the IRB and the FDA. The recipient will be followed for safety reason per protocol. The recipient will be replaced and the data analyzed separately.

Methotrexate in the Stem Cell Recipient: In accordance with standard practice of T-replete stem cell transplantations, in the event of a technical failure with a decision to proceed with a T-replete graft off study, methotrexate 5mg/m^2 will be given post-transplant on days 1,3 and 6 in addition to the cyclosporine already described in the protocol (Ringden et al., 1993).

Stem cell products will be cryopreserved after each collection and stored until transfusion into the recipient (see NIH Clinical Center, DTM, Cell Processing Section's SOPs). From experience with over 100 stem cell processing procedures, we anticipate that in most cases both the CD34+ cell target and the CD3+ T cell target will be achieved. In the majority of donors, peripheral blood stem cell processing with the Miltenyi system will result in doses of CD3+ cells well below 5×10^4/kg. In such cases the T cell dose will be adjusted to ensure that at least 5×10^4/kg are transfused with the allograft. The source of CD3+ T cells for the addback will be an aliquot from G-CSF mobilized lymphocytes.

The erythrocyte content of the apheresis product is approximately 1.0 ml of packed RBCs per liter processed. Therefore the typical RBC content of a 25-liter PBPC product would be about 25 ml. CD34 positive selection procedures results in elimination of RBCs and thawing will also lyse residual donor RBCs. Donor plasma will also be eliminated by the processing procedures. Therefore, minor ABO or other RBC incompatibility between donor and recipient, will require no specific processing or precautions during processing of the PBSC product. PBSC products will be cryopreserved after processing.

All products will be prepared for infusion by standard operating procedures of the DTM Cell Processing Section. Details for cell processing for this protocol are in the Protocol Specific Instruction (PSI) SOP document in Cell Processing. Detailed information is also provided by the Drug Master File BB MF 11054 "Facility, Operational and Quality Systems for Manufacture of HCT/Ps" of the DTM Cell Processing section (FDA registration #1174694). This includes the section's quality program, facilities, environmental control and monitoring, operational control systems and aseptic processing, equipments, supplies and eligibility, manufacturing systems, process development, process validation, process control and change control, product evaluation and lot release, storage, product labeling, label controls and tracking, product receipt and distribution, final product preparation, issue and administration, sterility testing, management of positive sterility tests, environmental impact and good laboratory practice statements.

8.6 Donor Monitoring and Follow Up Plan:

Donors will be contacted by our clinical staff within 48 hours after their cell collection. Adverse events will be documented and appropriate therapy will be provided either through the home physician or at the NIH. Donors will go off study one week after completion of their last apheresis collection or after resolution of any serious adverse events related to the collection, whichever is later. If additional cellular products are required at a later stage from the same donor, they will donate under the standard care protocol 94-H-0010.

9.0 MANAGEMENT OF COMPLICATIONS

The major complications are reactivation of cytomegalovirus (CMV), acute and chronic GVHD, leukemic relapse, and graft failure. Subjects with these complications will be treated as follows:

9.1 Viral Reactivation: Will be managed according with standard antivirals according to NIH BMT Consortium Supportive Care Guidelines.

Overwhelming Viral Infection: Additional unscheduled add-back of lymphocytes (a bulk DLI, please see section 7.12), may be given at any time if an indication arises such as viral infections, EBV lymphoproliferative disorder. Add-back may be given in the presence of GVHD, if the investigator considers the risk from overwhelming viral infection to outweigh the risk of exacerbating GVHD.

9.2 **Acute GVHD (**See NIH BMT Consortium Supportive Care Guidelines). GVHD markers have been recently validated in the clinical setting and will be utilized to guide therapy when available. Subjects developing steroid resistant or refractory GVHD may also be offered treatment with bone marrow stromal cells (MSCs) or other clinical trials.

9.3 **Chronic GVHD (**See also NIH BMT Consortium Supportive Care Guidelines**)**

- Cyclosporine at standard dose.
- Prednisone dosed according to severity.
- Change to alternate day steroid and cyclosporine therapy when response is established.
- Non-responding subjects may be treated with other standard of care therapies including but not limited to PUVA, extracorporeal photopheresis, low-dose IL2, mycophenolate, azathioprine, photopheresis, daclizumab, infliximab, rituximab, MSCs (on clinical trial), tyrosine kinase inhibitors or thalidomide at the discretion of the attending physician.

9.4 **Graft Failure:** Failure to achieve a neutrophil count of 200/µl by day 21 post-transplant and the aplastic appearance of a marrow sample taken 21-28 days after transplant will be deemed primary graft failure (also known as failure to engraft).

Delayed (secondary) graft failure after initial achievement of ANC recovery to >500/µL for three consecutive occasions is a recognized risk from T cell depleted transplantation. In such cases, loss of donor CD3+ chimerism precedes loss of donor myeloid chimerism. Donor CD3+ chimerism will be carefully monitored and falling donor CD3+ chimerism will be treated with escalating dose DLI +/- prior conditioning.
Stem cell rescue/boost from a CD34+ selected graft from the original donor may be necessary in cases of graft failure. This is permissible under the protocol after suitable preconditioning, typically fludarabine +cyclophosphamide + either ATG or Campath. However, stem cell boost for primary graft failure will declare the subjects as having reached an end of study criterion, and the subjects will at that point be taken off study and be offered a standard care protocol or an experimental protocol if available. *Note:* Stem cell boosts for secondary graft failure or other cytopenias will not represent an end of study event.

9.5 **Relapse of Hematologic Malignancy:** Markers for minimal residual disease will be incorporated, where available, into the management of recipients. Standard approaches to minimal residual disease monitoring include flow cytometry, PCR and array-based techniques, which are unique to each type of malignancy.

As the mortality for recipients relapsing before day +200 is very high, only salvage therapy is available. Any relapse will be considered a protocol end of study criterion. Subjects relapsing will be declared off-study and offered a standard care protocol or an experimental protocol for relapsed malignancy if available.

- Subjects with progressive disease after transplant requiring intervention or who are deemed to have relapsed will be managed as detailed in section 11.6.2.

- Subjects with detectable disease may receive pre-transplant chemotherapy as clinically indicated to decrease disease burden and increase the chance of successful transplantation as detailed section 7.1). The PI will decide on continuing the pre transplant therapy or initiating similar post-transplant antineoplastic therapy (such as rituximab for CLL, imatinib for CML, sorafenib for Flt3-ITD AML or other standard care treatments) on an individual basis (especially in the setting of detectable disease at or after transplant).

9.6 **Pulmonary Engraftment Syndrome:** Subjects who develop pulmonary engraftment syndrome (most likely 10-14 days post-transplant) will be treated with steroids.

9.7 **Delayed or Falling Donor Chimerism:** This will be detected by serial chimerism testing. DLI, with or without pretreatment by additional immunosuppressive drugs, may be given in the setting of poor chimerism, falling chimerism or delayed chimerism which places the subject at increased risk of relapse. Subjects showing poor engraftment unresponsive to standard treatments may be offered treatment with bone marrow stromal cell infusions on another applicable NHLBI protocol.

9.8 **Organ failure:** Subjects developing organ failure (pulmonary, GI, GU or hepatic) unresponsive to standard treatments may be offered treatment with bone marrow stromal cell infusions on clinical trial.

9.9 **Reactions to the graft and ABO incompatibility:** Instances of infusional reactions and ABO incompatibility are expected to be rare given the manipulation of the stem cell graft. For management please refer to NIH BMT Consortium Supportive Care Guidelines.

9.10 **Endothelial Damage Syndromes:** These comprise a spectrum of overlapping disorders such as transplant microangiopathy, posterior reversible encephalopathy syndrome, diffuse alveolar hemorrhage, idiopathic pneumonitis, sinusoidal occlusive syndrome/veno-occlusive disease and delayed cardiovascular disease. Management includes removal of triggers (calcineurin inhibitor/sirolimus) and defibrotide according to NIH BMT Consortium Supportive Care Guidelines.

9.11 **Cytokine release syndrome/cytokine storm:** This typically occurs early (engraftment syndrome) but may occur beyond 1 year. A triggering event such as viral infection or PTLD may be identifiable. The diagnosis is typically one of exclusion and the differential diagnosis includes infections and GVHD. Capillary leak (with anasarca, hypoalbuminemia, pre-renal azotemia, effusions, lung infiltrate) is a distinctive manifestation and may respond to IL6 blockade. Other findings are non-specific- cytopenias, coagulopathy, high fevers, transaminitis, delirium, cardiopulmonary, rash and organ failure. Characterization could involve CRP, ferritin, triglyceride, soluble CD25 receptor, IL1/TNF-a/IFN-g/IL6 levels, endothelial damage markers, coagulation parameters, imaging, marrow/skin biopsy. The severity of inflammation and clinical features may mimic that of secondary Hemophagocytic Lymphohistiocytosis(HLH) / Macrophage Activation Syndrome (MAS) and there may even be features of vasculitis on tissue biopsy. Management is supportive with anti-inflammatory therapy and treatment of an underlying trigger.

10.0 ANCILLARY LABORATORY RESEARCH STUDIES

10.1 Research Sample Collection

With Amendment Q, Sept 2017, research sample collection will cease.

During the course of participating on this study, specimens will be collected for correlative studies as follows:

10.1.1 Blood Sample from the recipient

Regular samples for research studies of engraftment, cytokines, lymphocyte subsets, malignancy antigen expression, minimal residual disease, graft-versus-leukemia effects will be collected as

below. Sampling may be cancelled at the discretion of the PI based upon the availability of the patient and the clinical status. The overall composition of the types and number of tubes used to collect research blood may be subject to alteration from time to time as long as there is no increase in the volume of blood or the timing of collection. All research studies will conform to those listed in the Appendix A, list of IRB approved Hematology Branch Research Studies. Note: per NIH guidelines, the amount of blood that may be drawn from adult patients and volunteers for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period. For pediatric patients, no more than 5 mL/kg may be drawn for research purposes in a single day, and no more than 9.5 mL/kg may be drawn over any eight-week period.

- *Preconditioning: 100 ml peripheral blood draw any time before starting conditioning (this blood draw replaces an apheresis session).*
- **Weekly peripheral blood draw (interval +/- 4days): Not to exceed 40 ml starting after day+ 14 through day+120or weight-based maximum. [* not collected on weeks of 120 mL collection]*
- *Days 30, 60, 90,120 blood draw (+/- 7 days): Not to exceed 120 ml or weight-based maximum*
- *6 month interval visits (+/- 3months): Not to exceed120 ml or weight-based maximum*

10.1.2 *Apheresis: One apheresis collection of approximately 10^{10} leukocytes in a volume of 200 ml, between 80-180 days post-transplant (when feasible per the PI).*

10.1.3 *Blood sample from the donor (Optional). 30 ml peripheral blood day 14, 21, 30 and 60 (when available). Donor leukocytes obtained at leukapheresis additional to those required for T cell add-back will be used for research studies investigating graft-versus-leukemia effects and testing new T cell depletion techniques. The recipient's leukemia cells and normal lymphocytes will serve as targets for all subsequent testing of donor-anti host responses.*

10.1.4 *Patient Bone Marrow Samples: An extra volume (up to 25 ml) of bone marrow aspirate will be collected for research at the same time as the pre transplant evaluation and when clinically indicated for monitoring minimal residual disease. The cells will be used to investigate lymphocyte interactions with bone marrow progenitor cell proliferation. No marrow aspirates solely for research purposes are planned.*

10.2 Collection, Storage and Disposition of Samples

Intended use: These specimens will not be read by a pathologist or used for diagnostic purposes. These studies will not be used in assessing the primary endpoint but are undertaken for descriptive or exploratory ancillary research, and have been approved by the NHLBI IRB and are listed in the Appendix A of the protocol. Research data is the confidential property of the generating party, hence all such information shall not be disclosed outside of NIH for any purpose other than as described in section 12.6.

Tracking: Samples will be ordered and tracked through the CRIS Research Screens. Should a CRIS screen not be available, the NIH form 2803-1 will be completed and will accompany the specimen and be filed in the medical record. Samples will be processed and stored in the laboratory of Dr. John Barrett and will not be sent outside NIH without IRB notification and an executed MTA.

Storage: Research samples will be stored with identifiers in the secure laboratory of the Dr. John Barrett using the NHLBI's BSI tracking system.

End of study procedures: Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

Loss or destruction of samples: Should we become aware that a major breech in our plan for tracking and storage of samples has occurred, the IRB will be notified.

11.0 BIOSTATISTICAL CONSIDERATIONS

11.1 Primary Endpoint and Primary Hypothesis

The primary endpoint of this protocol is to determine the rate of overall survival at 200 day using the Miltenyi CliniMACS CD34selection system. Thus, the primary endpoint is all cause mortality at day 200, and the proportion of subjects who have survived at day 200 will be used to determine the sample size. We have consistently used the day 200 overall survival endpoint for serial T cell depletion trials in the Hematology Branch, enabling us to identify potential problems early. The primary hypothesis is that the 200-day survival rate for subjects treated with allogeneic transplant using the Miltenyi CliniMACS CD34selection system will exceed 75%.

11.2 Secondary Endpoints

Secondary endpoints will include standard transplant outcome variables such as relapse, non-relapse mortality, disease free survival, engraftment kinetics of donor neutrophils, platelets and lymphocytes, incidence and severity of acute GVHD and chronic GVHD (limited and extensive) and GVHD-free survival. Technical failure rates and the use of DLIs will be tracked as will concurrent participation on adoptive antiviral/antimalignancy cellular therapy protocols. Outcomes of transplants in subjects with advanced age will be tracked.

Subjects who co-enroll onto any antiviral adoptive cellular therapy protocol (under IND# 16061), will continue to have their primary and secondary endpoints measured on 13-H-0144 and will not be "removed". Also, since this is an objective of the 13-H-0144 protocol, we will compare primary and secondary endpoints in patients receiving the antiviral adoptive cellular therapy versus those who do not for various reasons such as: a. belonging to the first cohort of ~11 subjects who did not receive antiviral cellular therapy b. refusal to receive antiviral lymphocytes c. death prior to administration of antiviral lymphocytes or d. technical failure of manufacture and/ or release of the antiviral lymphocyte product. We also have the option of including in this comparison subjects on our prior CD34+ selected transplant trial (concluded protocol 06-H-0248, under this same IND), none of whom received antiviral lymphocytes.

11.3 Sample Size

Existing result in the literature suggests that the 200-day survival rate for subjects treated with T-cell depleted PBSCT should be at least 75%, and our treatment would be determined to be ineffective if the 200-day survival rate under this protocol is not higher than 75%. Our experience in protocol 06-H-0248 also suggests that the 200-day survival rate may be as high as 86%. Thus, we consider here the Simon's "Optimum Two-Stage" design for testing the null hypothesis H0: "the 200-day survival rate is at or below 75%" versus the alternative H1:"the 200-day survival

rate is 86% or higher" with 0.05 significance level and 0.80 power. This design has the advantage of allowing us to stop the study early at the first stage if the true 200-day survival rate is likely to be 75% or lower. Based on this design, we will accrue 31 patients in the first stage, and the trial will be continued to the second stage if 25 or more patients are to survive to day 200. Otherwise, the trial will be stopped and the treatment will be determined ineffective, i.e., the null hypothesis H0 will be accepted. If the trial goes on to the second stage, a total of 96 patients will be accrued, and the treatment will be determined ineffective, i.e. the null hypothesis H0 will be accepted, if the total number of patients to survive to day 200 is less than or equal to 78. If the treatment is actually not effective, i.e., the null hypothesis is true; there is a 0.046 probability of concluding it is, i.e., accepting the alternative hypothesis. On the other hand, if the treatment is actually effective, we have a 20% probability of concluding that it is not. The computation is carried out using the R Package "clinfun" version 1.0.5.

11.4 Methods of Statistical Analysis

Although the primary endpoint for this protocol occurs at 200 days, recipients will be followed up for 36 months on protocol and then indefinitely on the long term follow up protocol (05-H-0130) so that the incidence of chronic GVHD, long-term disease-free and overall survival can be estimated. At the completion of follow-up for all subjects, time-to-event distributions (disease-free survival, non-relapse mortality and overall survival) will be estimated using the Kaplan-Meier method and 95% confidence intervals for the median time to events will be calculated (T. Therneau and P. Grambsch, "Modeling Survival Data, Extending the Cox Model", Ch. 2, 2000, Springer: New York). When deemed appropriate, regression models in survival analysis, such as the Cox Proportional Hazard model, competing risk models and frailty models, will be used to evaluate the effects of covariates, such as age, treatment group, disease, baseline history of prior transplant, use of DLI in response to infection on time-to-event distributions (T. Therneau and P. Grambsch, "Modeling Survival Data, Extending the Cox Model", Ch. 3&8, 2000, Springer: New York). Logistic regression models (or other generalized linear models when deemed appropriate) will be used to evaluate the effects of covariates on the event rates of graft failure, chronic GVHD, leukemia relapse, NRM and other secondary endpoints.

Subjects will not be replaced however their outcome will be analyzed separately if they receive a final product that is designated a technical failure of the selection process (i.e. if the final CD3+ dose is greater than 5 x 10^5/kg CD3+ after reducing the CD34+ dose to 3 x 10^6/kg) (see section 7.7)

11.5 Dose Limiting Toxicity, Interim Analyses and Stopping Rules

Dose limiting toxicity is defined in terms of non-relapse mortality (NRM), a useful composite and early endpoint for overall safety. For safety NRM at day +200 will be monitored. To account for relapse post-transplant we will also analyze overall survival at day +200. Overall survival reflects death from relapse and NRM. Subtracting NRM from overall survival provides an estimate of relapse since most subjects die after relapse of disease. Although, not all subjects destined to relapse will have done so by day +200, most relapses do occur by then. The early estimate of relapse on day +200 will particularly detect a tendency towards precocious relapse caused by the protocol.

Interim analysis for optimization of engraftment and GVHD prior to any adoptive cellular immunotherapy

The intended use of this trial is as a stable, low-GVHD platform for adoptive immunotherapy. Engraftment and GVHD rates will be analyzed on an ongoing basis. Since lymphocyte depletion increases the risk of slow donor CD3+ engraftment, adjustments in the dose of T cells added back on day 0 may be necessary. Once the T cell adjustment is finalized, the last block of 10 consecutive subjects will be analyzed. Future adoptive cellular transfer protocols will not start until the cohort of 10 consecutive recipients meet the following criteria:

- Primary graft failure (ANC<200/μL at day 21) in no more than 1 subject ***AND***
- Complete (>95%) donor CD3+ lymphocyte chimerism achieved at no later than a median of 180 days post-transplant ***AND***
- Acute GVHD (grade II-IV) <40%***AND***
- Formal IRB approval prior to adoptive cellular therapies.

As of May, 2014, this set of criteria has been successfully satisfied in the first 10 subjects

Parameter	***Study results in first 10 patients***	***Criterion for success***
Primary graft failure rate (ANC<200/μL at day 21)	ZERO	no more than 1 subject
Complete (>95%) donor CD3+ lymphocyte chimerism (median)	37 days	no later than 180 days
Acute GVHD (grade II-IV)	30%	<40%

With amendment version 6-5-14, this protocol will now permit enrolled subjects to participate in adoptive cellular therapy protocols."

Interim Analysis for Futility

One interim analysis is planned based on overall survival after the first 31 subjects have been transplanted, section 11.3. In order to proceed to the second stage, 25 out of these 31 subjects need to survive beyond day 200.

Stopping Rules for Safety (Early Stopping Rule- Non-relapse mortality at day 200 & Late Stopping Rule for Disease free survival at 2 years)

To prevent the possibility of having an unacceptable rate in early non-relapse mortality (NRM) for the study, NRM at day 200 will be monitored as a safety endpoint. Early stopping of the study will be considered if the numbers of NRM cross some established stopping boundaries. We adopt the Bayesian approach of Geller et al. (2004) (Nancy L. Geller, Dean Follmann, Eric S. Leifer, and Shelly L. Carter, "Design of Early Trials in Stem Cell Transplantation: A Hybrid Frequentist-Bayesian Approach" in Advances in Clinical Trial Biostatistics (Editor: Nancy L. Geller), 2004. New York: Marcel Dekker, Inc.) that formally incorporates our "prior" expectations about these rates. Based on experience with similar protocols, we assume that the 200-day NRM rate should not be higher than 20%. A stopping boundary is reached if the true rate exceeds the above anticipated rate with probability .90 or greater. We take our prior distribution to be the beta distribution with (alpha, beta) = (2, 8), so that alpha+beta= 10 is approximately 10% of the total

sample size. In order to prevent premature termination of the study, we will start safety monitoring when there are 4 deaths. The stopping boundaries are given in the table 3 below.

Table 3: Early stopping rule for safety based on NRM at day 200

Number of transplant recipients	"Stop" if # of NRM by day-200 reaches or exceeds
≤7	4
≤11	5
≤15	6
≤19	7
≤23	8
≤27	9
≤31	10
≤36	11
≤40	12
≤44	13
≤48	14
≤53	15
≤56	16
≤61	17
≤66	18
≤70	19
≤74	20
≤79	21
≤83	22
≤87	23
≤92	24
≤96	25

We investigated the performance of the above stopping rule by a simulation study. In each simulation run, we generated a study with 96 independent Bernoulli trials, each had a probability p for having NRM and q=1-p for not having NRM, and compared the NRM outcomes with the above stopping boundary to determine whether the study was stopped. We repeated the simulation 100,000 times and computed the proportion of stopped studies (i.e. "number of stopped studies"/100,000) which were stopped using the above stopping rule. The following **Table 4** summarizes the proportions of stopped studies under a number of p values:

Table 4: Proportion of stopped studies for different p values

Prob of NRM = p	5%	10%	15%	20%	25%	30%	35%
Proportion of Stopped Studies	0.03%	0.7%	5.7%	26.2%	64.2%	90.8%	98.9%
Average # of Subjects Needed	95.98	95.42	91.83	79.71	57.66	36.54	23.30
Average # of NRM	4.79	9.53	13.78	15.94	14.43	10.96	8.15

These results suggest that the above stopping rule has a low probability of stopping a study when the proportion of NRM is below the benchmark value of 20%, while the probability of stopping a study is high when the true proportion of NRM exceeds this benchmark value. Based on these results, we believe that this Bayesian stopping rule has satisfactory statistical properties.

Our past protocol 06-H-0248 suggests that the probability of serious late complications (defined as relapse or death, or 1-Disease-free survival) at 2 years is 49.5%. We quantify this experience by establishing a stopping boundary table for "2-year Relapse-or-Mortality" (RM) using a Bayesian approach based on the beta prior distribution with parameters (a, b) = (2.94, 3.06). We will immediately report to the DSMB and seriously consider stopping the study if the posterior probability that the underlying rate of "2-year Relapse-or-Mortality" exceeding 49% is at least 80%. The parameter are chosen so that the mean a / (a + b) = 49% as the expected proportion of SAEs and the relative weight we place on our prior opinion is about 10% of the weight we will place on the results of the new study of sample size 96. The stopping boundary is summarized in **Table 5** and early stopping of the study will be considered if the boundary is reached.

Table 5: Late stopping rule for safety based upon 2 year Relapse-or-Mortality

# of transplant recipients	Stop if # of recipients with relapse or mortality at 2 years reaches
≤ 6	5
≤ 8	6
≤ 10	7
≤ 12	8
≤ 14	9
≤ 16	10
≤ 17	11
≤ 19	12
≤ 21	13
≤ 22	14
≤ 25	15
≤27	16
≤29	17
≤31	18
≤33	19
≤35	20
≤37	21
≤38	22
≤40	23
≤42	24
≤44	25
≤46	26
≤48	27
≤50	28
≤52	29
≤54	30
≤56	31
≤58	32
≤60	33
≤62	34
≤64	35
≤66	36
≤67	37

# of transplant recipients	Stop if # of recipients with relapse or mortality at 2 years reaches
≤69	38
≤71	39
≤73	40
≤75	41
≤77	42
≤79	43
≤81	44
≤83	45
≤85	46
≤87	47
≤89	48
≤91	49
≤93	50
≤95	51
≤96	52

Table 6: Proportion of stopped studies for different probabilities of Relapse/Mortality

Prob of RM = p	30%	40%	50%	55%	58%	60%	65%
Proportion of Stopped Studies	2.8%	15.5%	58.7%	84.1%	93.4%	96.9%	99.8%
Average # of Subjects Needed	93.57	83.55	54.60	36.23	27.17	22.4	14.73
Average # of RM	28.07	33.42	27.28	19.91	15.75	13.5	9.58

We conducted a simulation study to evaluate the statistical properties of the stopping boundaries of Table 5 and summarized the simulation results in Table 6. Similar to Table 4, the simulation results in Table 6 suggest that the above stopping rule has a low probability of stopping a study when the proportion of RM is below the benchmark value of 49.5%, while the probability of stopping a study is high when the true proportion of RM exceeds 49.5%.

The DSMB will also evaluate all treatment related serious adverse events (TRSAEs) and will have access to treatment allocation. The DSMB may recommend early study termination of the trial if other unforeseen adverse events necessitate this decision.

11.6 Off Study Criteria

11.6.1 Withdrawal per subject choice

Recipients and their donors will be given ample opportunity to withdraw from the study prior to admission for conditioning and transplantation. Should they wish to withdraw prior to transplant they will be replaced in order to maintain appropriate statistical power to evaluate primary endpoints. After the transplant, the risks of withdrawal will be carefully explained. If the recipient chooses to withdraw before the primary endpoint of the study is reached, this recipient will be followed in the safety analysis.

Without compromising their participation in the treatment protocol recipients and donor may

at any time withdraw from participation in the ancillary laboratory research aspects of the protocol. Research data and samples collected prior to withdrawal of consent may still be used for analysis.

11.6.2 Withdrawal by physician decision

Recipients with disease **relapse** will be offered co-enrollment onto the standard of care protocol on which they may receive tapering of immunosuppression, donor lymphocyte transfusions, immunostimulation (i.e. interferon-alpha, IL-2, GM-CSF, rituximab, imatinib and/or chemotherapy) with or without stem cell rescue, or enrollment in phase I/II trials, or they may be referred back to their referring physician (whichever is in the best interest of the patient). Data collection to include outcome and survival will continue, however complications following standard of care therapies will be reported per the standard of care protocol.

Recipients who fail to achieve hematological recovery (**primary graft failure**) will be offered co-enrollment onto the Branch's standard of care protocol where they may be treated with further immunosuppression, growth factors or a second stem cell transplant using unmanipulated G-CSF mobilized, donor stem cells or they may be referred back to their referring physician whichever is in the best interest of the patient. Data collection to include outcome and survival will continue, however complications following standard of care therapies will be reported per the standard of care protocol.

If the **target dose of CD34+ and CD3+ cells cannot be achieved** after processing all the collections, the recipient will go off study **(technical failure)** and be offered the choice of participating in other protocols or referral back to their home physician. In this event, the recipient will be replaced in order to maintain appropriate statistical power to evaluate primary endpoints.

Subjects with a **significant decline in performance status** (e.g. life-threatening GVHD) which negates further treatment on protocol will be offered co-enrollment onto the standard of care protocol or referred back to their home physician depending on what is in the best interest of the patient. In such cases, typically at end of life, where it is impossible to follow protocol mandated investigations and treatments, the patient will remain on protocol for collection of endpoints but the standard care provided will not be considered a protocol deviation. Data collection to include outcome and survival will continue, however complications following standard of care therapies will be reported per the standard of care protocol.

11.6.3 Completion of the study.

Following the completion of 200 days of follow up care the recipient and donor will go off study. At 3 years after transplantation, the subjects will be offered the opportunity to enroll on the Late Effects of Transplant protocol "*Long-term evaluation and follow up care of patients treated with allogeneic stem cell transplants*" (05-H-0130). This late effects protocol is designed to evaluate the very late consequences of transplant treatment and, if necessary, manage transplant recipients suffering from iatrogenic consequences of treatment. Recipients opting not to continue follow up care on the Late Effects protocol will be referred back to their primary care physician.

12.0 DATA AND SAFETY MONITORING

12.1 Safety Monitoring

Principal Investigator: Accrual and safety data will be monitored by the PI. The protocol will be continuously evaluated for any unusual or unpredicted complications with the aim of detecting and preventing unacceptable increase in morbidity and mortality over and above that anticipated from unmanipulated bone marrow transplants (see stopping rules in section 11.5).

NHLBI IRB: Prior to implementation of this study, the protocol and the proposed recipient and donor consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating in accordance with 45 CFR 46. The IRB will conduct continuing annual review of accrual and safety data. IRB approval will be obtained for all amendments to the protocol or informed consent for as long as the protocol is open to accrual or follow up of subjects.

***DSMB*:** The NHLBI Data Safety and Monitoring Board (DSMB) will review the protocol at the regularly scheduled six or twelve month intervals as applicable. A progress report will be forwarded to the DSMB at these times. The DSMB may recommend early termination of the study for considerations of safety and efficacy.

Miltenyi Biotec, Inc.: An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to Miltenyi Biotec, Inc. to

Tara Clark, General Manager US Clinical Operations
Miltenyi Biotec, Inc.
85 Hamilton Street Cambridge, MA 02139- 4524

FDA*:* An annual progress report, any amendments to the protocol, and any change in the status of the protocol will be forwarded to FDA to:

U.S. Food and Drug Administration
Center for Biologics Evaluation and Research
Document Control Center
10903 New Hampshire Avenue
WO71, G112
Silver Spring, MD 20993-0002

12.2 Adverse Event Reporting

Adverse events used to evaluate the safety of this protocol regimen will be collected to include any unfavorable and unintended signs (including abnormal laboratory findings), symptoms and/or diseases (i.e. incidence of GVHD, graft failure, regimen related toxicities, or infectious complications) which either occur during the study, having been absent at baseline or if present at baseline, appear to worsen with the following exceptions. The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and/or disease and graded by severity utilizing CTC version 3.0. A copy of the criteria can be down-loaded from the CTEP home page at http://ctep.cancer.gov/reporting/ctc.html.

Adverse events considered possibly, probably, or definitely related to investigational agents administered as adoptive cell therapies in conjunction with this protocol will be reported only under the clinical protocol for the stated cell therapy.

12.2.1 Definitions

Adverse Event (AE): Any untoward medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

Serious Adverse Event (SAE): A serious adverse event that:

- results in death;
- is life-threatening (places the subject at immediate risk of death from the event as it occurred);
- results in in-patient hospitalization or prolongation of existing hospitalization;
- results in a persistent or significant incapacity;
- results in a congenital anomaly/birth defect; or
- based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition.

Serious event: An event is serious if it meets the definition of a serious adverse event (above) or if it requires immediate corrective action by a PI and/or IRB to protect the safety, welfare or rights of subjects.

Unanticipated Problem (UP): Any incident, experience, or outcome that meets <u>all</u> of the following criteria:

1. **unexpected** in terms of nature, severity, or frequency in relation to
    a. the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents; and
    b. the characteristics of the subject population being studied; and
2. **related or possibly related** to participation in the research; and
3. places subjects or others at a **greater risk of harm** (including physical, psychological, economic, or social harm) than was previously known or recognized.

Unanticipated Problem that is not an Adverse Event: An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involves risk to the subject, affect others in the research study, or significantly impact the integrity of research data. For example, report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

Protocol Deviation (PD): Any change, divergence, or departure from the IRB approved research protocol.

Event Characterization and Reporting to the IRB and Clinical Director (CD)
Approved by HSRAC on September 30, 2013
Date effective: October 28, 2013

All adverse events occurring during the study, including those observed by or reported to the research team, will be recorded. Serious unanticipated problems, and serious protocol deviations, will be reported to the IRB and Clinical Director as soon as possible but not more than 7 days after the PI first learns of the event. Not serious unanticipated problems will be reported to the IRB and Clinical Director as soon as possible but not more than 14 days after the PI first learns of the event. Not serious protocol deviations will be reported to the IRB as soon as possible but not more than 14 days after the PI first learns of the event. SAEs that do not meet the criteria of Unanticipated Problem (UP) must be reported to the IRB Chair and Clinical Director within 14 days of learning of the event.

Deaths will be reported to the Clinical Director within 7 days after the PI first learns of the event.

12.3 Adverse Events (AEs)–Transplant Recipients

The following are expected outcomes for the transplant recipient and will be documented in the subjects medical record but not reported to IRB unless they meet the criteria for an SAE as defined in Section 12.2.1

- Renal insufficiency
- Hepatic insufficiency
- Transient cardiac arrhythmias
- Transient cardiac insufficiency
- Pulmonary insufficiency
- Neutropenia and its complications
- Thrombocytopenia and its complications
- Anemia and its complications
- Transfusion reactions
- Treatable infections from bacteria, viruses, protozoa and fungi
- Late effects of transplant regimens including: chronic fatigue, cataracts, infertility, growth impairment, hypothyroidism, bone complications, dental caries, cardiovascular, delayed infections, subsequent neoplasms
- Headache, insomnia, psychosis, mood changes, disorientation, seizures from metabolic imbalance
- Nausea, vomiting, diarrhea, mucositis, weight loss, dry mouth, hiccoughs, constipation
- Well-characterized drug reactions - allergic manifestations, "red man" syndrome, steroid effects
- Well-characterized drug side effects from drugs used routinely in transplant recipients (e.g.; preparative regimen chemotherapy, immunosuppressive drugs, antimicrobials)
- Common side effects of antiemetics, analgesics, anti-inflammatory agent and known complications of steroid therapy
- Complications from intravenous catheters, thrombotic occlusion, infection, local reactions, cardiac arrhythmia

- Expected adverse events related to investigational reagents and transplant drugs are listed in section 13.3.

The following are expected transplant outcomes that will be reported in summary form at the time of continuing review but will not be reported to IRB at each occurrence unless they meet the criteria of an SAE as defined in section 12.2.1:

- Acute graft-versus-host disease
- Chronic graft-versus-host disease
- Graft failure / graft rejection
- Veno-occlusive disease
- Hemorrhagic cystitis
- Cytomegalovirus reactivation or disease
- EBV reactivation or disease
- Autoimmune phenomenon
- Fungal infections
- Disease relapse or progression

12.4 Adverse Events - Donors

The following are expected outcomes for the donor that will not be reported to the IRB unless they meet the criteria of an SAE as defined in section 12.2.1:

- Common side effects of filgrastim (G-CSF) administration (bone pain, fatigue, arthralgias, headache, insomnia, fever, worsening of pre-existing skin rashes, increases of alkaline phosphatase, lactate dehydrogenase and/or uric acid levels, elevated blood leukocyte count, or thrombocytopenia)
- Hypotension during apheresis
- Hospital admission to safeguard a catheter

The following are expected outcomes for the donor that will be reported in summary form at the time of continuing review but will not be reported to IRB at each occurrence unless they meet the criteria of an SAE as defined in section 12.2.1:

- Ischemic chest pain during filgrastim (G-CSF) administration
- Splenic enlargement
- Cutaneous vasculitis
- Bone pain, muscle aches or headaches requiring narcotic analgesics

12.5 Reporting of SAEs

All serious adverse events will be reported to the Principal Investigator

Minoo Battiwalla, M.D., M.S.
Bldg 10, CRC Room 5-3581
Phone: 301-827-0939
Fax: 301-827-3228

The Principal Investigator, Minoo Battiwalla, M.D., M.S. shall submit to the sponsor, A. John Barrett, M.D. a report of any unanticipated adverse device effect occurring during an

investigation as soon as possible, but in no event later than 10 working days after he first learns of the effect.

IRB: Suspected Unexpected Serious Adverse Reaction (SUSAR) and any unanticipated adverse device effectobserved during the clinical trial for which there is a relationship with the use of the Miltenyi CliniMACS CD34Selection System and/or its components or the conduct of the study will be reported to the IRB as soon as possible, but in no event later than 10 working days, except for a life threatening or fatal outcome which will be reported within 7 days.

Miltenyi Biotec, Inc.: Reports of SUSARs and any unanticipated adverse device effectobserved during the clinical trial and for which there is a relationship with the use of the Miltenyi CliniMACS CD34Selection System and/or its components will also be forwarded as soon as possible to Miltenyi Biotec. Miltenyi will be provided with a copy of the annual FDA progress report which includes a summary of all SAEs occurring in this clinical trial.

Tara Clark, General Manager US Clinical Operations
and Norman Pilon, PhD
Miltenyi Biotec, Inc.
85 Hamilton Street
Cambridge, MA 02139- 4524

FDA*:*
IDE Unanticipated Adverse Device Effects Report (Refer to 21 CFR 812.50): The IDE sponsor, A. John Barrett, M.D., will report SUSARs and any unanticipated adverse device effect observed during the clinical trial for which there is a relationship with the use of the Miltenyi CliniMACS CD34 Selection System and/or its components on the conduct of the study to the FDA as soon as possible, but in no event later than 10 working days after the sponsor learns of the effect. Thereafter, the sponsor shall submit such additional reports concerning the effects as FDA requests.

FDA Annual Reports (Refer to 21 CFR 812.150)
The study sponsor will submit progress reports at regular intervals and at least annually to the FDA. All communications to the FDA will be submitted to:

U.S. Food and Drug Administration
Center for Biologics Evaluation and Research
Document Control Center
10903 New Hampshire Avenue
WO71, G112
Silver Spring, MD 20993-0002

DSMB
SUSARs and any unanticipated adverse device effectobserved during the clinical trial and for which there is a relationship with the use of the Miltenyi CliniMACS® CD34Selection System and/or its components or the conduct of the study will also be reported within 24 hours to the Data and Safety Monitoring Board (DSMB). A summary of all SAEs and AEs will be included for review at the regularly scheduled DSMB meeting.

12.6 Data Management

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. Laboratory values from referring home physicians will be entered into the system. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts to ensure that data is verifiable and evaluable.

Research data will be prospectively collected by authorized Investigator personnel and entered into a NHLBI Database.

The database will maintain complete data records on each research subject. Subjective and objective patient experiences during the duration of the study will be documented in the patient medical record notes. These protocol notes will serve as the primary source material from which data will be collected and research analyses will be performed. Any pertinent supplementary information obtained from outside laboratories, outside hospitals, radiology reports, laboratory reports, or other patient records will be used as additional source for data collection.

Neither individual personal identifiers nor the key linking coded data to individuals will be released to Miltenyi Biotec, Inc. without prior IRB approval and an executed MTA or CTA.

End of study procedures: Data will be stored in locked cabinets and in a password protected database until it is no longer of scientific value. Identifiable data will not be sent outside NIH without prior IRB approval or appropriate conditions for disclosure outlined in the executed MTA or CTA. After closure of this protocol at this time as all primary endpoints for this study have been met, all remaining samples and data will be transferred to protocol 17- H-N001 "Retrospective Data and Biospecimen analysis for Allogenic Stem Cell Transplantation Protocols". The project covers existing data review and biospecimen analysis from allo-SCT protocols performed at the Hematology Branch, NHLBI, NHLBI.

Loss or destruction of data: Should we become aware that a major breech in our plan to protect patient confidentiality and trial data has occurred, the IRB will be notified.

CIBMTR: For the purposes of quality assurance (i.e. accreditation of the Transplant program), data will be released to the Center for International Blood and Marrow Transplant Research (CIBMTR) in accordance with federally mandated policies and procedures.

Publication Policy: Given the research mandate of the NIH, patient data including the results of testing and responses to treatment will be entered into an NIH-authorized and controlled research database. Any future research use will occur only after appropriate human subject protection institutional approval such as prospective NIH IRB review and approval or an exemption from the NIH Office of Human Subjects Research Protections (OHSRP).

All of the following elements will be recorded in the database and subject to updates by the PI:

A. Subject Enrollment

- Date of birth, age, gender, race, ethnicity, MRN
- Height, Weight, Performance Status (ECOG AND Karnofsky)
- Disease diagnosis, subdiagnosis, date of original diagnosis
- Stage at diagnosis and at study entry

- HCT-CI score, EBMT score pre transplant
- Disease morphology (marrow) at diagnosis, at last progression and at study entry along with dates
- Cytogenetics/FISH at diagnosis, at last progression and at study entry along with dates
- Molecular testing at diagnosis, at last progression and at study entry along with dates
- Prior therapy for disease
- Number of prior transplants for recipient
- Recipient: PPD test results, serologies for CMV, EBV, Hepatitis A,B,C, HIV, HTLV, Toxoplasma, RPR.
- Recipient: Echocardiogram (LVEF), Pulmonary Function Testing
- Donor and recipient: CBC and comprehensive metabolic panel, ferritin, CRP.
- Donor/and recipient : HLA typing, KIR typing
- Donor/and recipient : Date of Informed Consent signature, consent version and date of registration
- Donor/and recipient : CMV serostatus, ABO-RH types,
- Donor: gender, gravida//parity if female, relationship to recipient, date of birth, age, gender, race, ethnicity, MRN
- Donor: serologies for CMV, EBV, Hepatitis A,B,C, HIV, HTLV, Toxoplasma, RPR, T.cruzii, W. Nile.

B. Cellular product administration

- Date of stem cell infusion (transplant date)
- CD34+ dose (x10e6/kg), CD3+ dose (x10e4/kg)
- Given per protocol: G-CSF mobilized PBPC, CD34+/kg is >3x10e6 on D0, CD3+/kg is 5x10e4 to 1x10e6 on D0, Fludarabine [25 mg/m2/d x 5 on days -8 to -4], Cytoxan [60 mg/kg x2 on days -3 and -2], Total Body Irradiation [<55yrs: 8 x 1.5 Gy, BID days -7 to -4 w lungs shielded OR ≥55 yrs 8 x 0.75 Gy& no lung shielding], CSA from D-6 to D+21
- Post-transplant therapy (Imatinib or other TKI, FGF, Palifermin)
- Cyclosporine duration- at least 21 days.

C. Engraftment

- ANC and platelet recovery
- Primary or secondary graft failure
- Time to donor CD3+ chimerism >95%
- Time to donor myeloid chimerism >95%
- Engraftment Syndrome requiring steroids

D. GVHD

- Acute GVHD: maximum grade (CIBMTR definition), date of first onset
- Chronic GVHD: Maximum grade (Shulman criteria), date of first onset
- Last date of systemic immunosuppression

E. Infectious complications

- Date of first CMV >500 copies
- CMV organ disease- site/date
- First CMV/ EBV reactivation requiring treatment (site/date)
- Proven viral hemorrhagic cystitis (type Adeno/BK, date)
- Fungal infections- types, site, date
- Blood stream/vital organ/severe manifestation/opportunistic organism infection

F. Second malignancy

- Date, type, EBV positivity, donor vs recipient origin cell

G. Severe pulmonary toxicity

- Defined as requiring mechanical ventilation, hypoxia with SaO2<90% on RA, Any BAL or lung biopsy, any lung GVHD (COP or BOS).
- Date, type and therapy.

H. Other toxicity

- Suspected Thrombotic microangiopathy and date
- Veno occlusive disease and date
- Other serious complication (CTCS-AE grade4 and above), type, date, treatment.

I. Donor cellular Infusions

- Type of cells, Cell dose, indication, pretreatment regimen, Acute GVHD date, Acute GVHD maximum grade

J. Malignant disease evaluation:

- First relapse/progression: date, molecular staging, cytogenetic/FISH staging, Morphology, Imaging staging with dates.
- All restaging evaluations: molecular staging, cytogenetic/FISH staging, Morphology, Imaging staging with dates.
- Additional antimalignancy treatment- chemotherapy, immunotherapy, donor cellular infusions, radiation

K. Vital outcomes;

- Last contact date
- Alive/dead, date of death.
- Subject accrued but did not start conditioning
- End of study: date, reason, comments
- Cause of death, date of death, subtype of non-relapse mortality
- Autopsy findings

L. Adverse Events

- Regimen Related Toxicity (Bearman scale)
- Serious Adverse Events

12.7 Protocol Monitoring

As per ICH-GCP 5.18 and FDA 812.46 clinical protocols are required to be adequately monitored by the study sponsor. The monitoring of this study will be conducted by Clinical Research Associates (CRAs)/Monitors employed by an independent contract organization working under an agreement with NHLBI to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent form (ICF) and documentation of the ICF process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents

(subject's charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms and pertinent hospital or clinical records readily available for inspection by the local IRB, the FDA, the site monitors, and the NHLBI staff for confirmation of the study data.

13.0 HUMAN SUBJECT PROTECTIONS

The investigators will protect the rights and welfare of human research subjects set forth in 45 C.F.R. Part 46 and 21 C.F.R Part 50, *Protection of Human Subjects.*

13.1 Rationale for Subject Selection

This protocol is open to males and females from all ethnic and racial groups. Distribution of transplant recipients on prior hematology branch protocols 06-H-0248 and 12-H-0028 was as follows:

- by disease: 100% neoplastic conditions. 41% AML; 29% ALL; 17% MDS/MPD/CMMoL; 7% NHL/CLL; 4% CML; 1% biphenotypic acute leukemia.
- by gender: 50.7% females; 49.3% males
- by age: median age 42, range 8 to 69.
- by race: 5.3% Asian, 9.3% Black, 37.3%, White, 48% Unknown or other.
- by ethnicity: Hispanic or Latino 53.3%; Non-Hispanic 46.6%.

Recruitment: The study will be listed on clinicaltrials.gov, clinical center research studies, PDQ, Aplastic Anemia Foundation, and the NHLBI patient recruitment websites. If recruitment goals are not met, a recruitment plan will be developed by the Clinical Center Office of Patient Recruitment.

Competition with other Branch transplant protocols:

03-H-0170: Non-myeloablative Allogeneic Peripheral Blood Mobilized Hematopoietic Precursor Cell Transplantation for Hematologic Malignancies in High Risk Subjects and in Subjects with Debilitating Hematologic Diseases

02-H-0250: A Phase I/II Study of HLA-matched Mobilized Peripheral Blood Hematopoietic Stem Cell Transplantation for Advanced Mycosis Fungoides/Sezary Syndrome Using Nonmyeloablative Conditioning with Campath-1H.

08-H-0186: Safety and the Anti-Tumor Effects of Escalating Doses of Adoptively Infused Ex Vivo Expanded Autologous Natural Killer (NK) Cells Against Metastatic Cancers or Hematological Malignancies Sensitized to NK-TRAIL Cytotoxicity with Bortezomib

Reimbursement for protocol participation, travel, food, and lodging

There is no payment or compensation to subjects for their participation in this protocol.

Reimbursement for travel, food, and lodging will be consistent with NHLBI DIR Travel and Lodging Compensation of Clinical Research Subjects policy or institutional guidelines.

13.2 Participation of Children

13.2.1 As recipients:

Pediatric subjects under 10 years of age are excluded from participating in the protocol because in children less than age 10, the risk of severe GVHD (one of the secondary endpoints of the study) is substantially less than that experienced in transplant recipients over the age of 10. It is therefore, not appropriate to expose children under the age of 10 to an experimental protocol designed to evaluate the incidence and severity of acute GVHD after transplant.

13.2.2 As donors:

We are excluding from participation as donors, children who weigh $\leq$18 kg and are < 2 years of age. The risks of the apheresis procedure are related to the weight of the child, more precisely his/her extracorporeal volume, which is weight-dependent. The risks have to do with (1) need for a central line, (2) need for an allogeneic red cell prime, (3) need for systemic heparinization because subject is too small to get citrate:

25 kg: the procedure and associated risk is the same as that in an adult, however a central line is often needed (at the discretion of the apheresis department).

19 to 25 kg: A central line is usually required (at the discretion of the apheresis department). With concurrent magnesium and calcium infusion, children may be safely anti-coagulated with citrate.

Two apheresis procedures will be performed in minor donors - one for the collection of lymphocytes and the other for the collection of stem cells. Collecting stem cells and lymphocytes in two apheresis sessions is widely practiced in donors of all ages including children *(Elmaagcli et al 2003, Barge et al. 2003, Reviewed in Baron and Beguin 2002)* DLIs may be given post-transplant to treat relapse, to treat recurrent infection, or to promote engraftment of stem cells when at risk of rejection after a SCT regardless of how the stem cells are prepared.

13.2.3 *As participants in research studies:*

Pediatric donors may participate only in those laboratory studies that the IRB finds involves no greater than minimal risk to children provided that adequate provisions are made for soliciting the assent of the children and the permission of their parents or guardians.

13.3 Hazards and Discomforts

13.3.1 The recipient

Related to the transplant procedure:

Blood and marrow stem cell transplantation is a major procedure, which entails serious discomforts and hazards for the patient. The mortality from the transplant procedure itself may be

as high as 40%. It is therefore only appropriate to carry out the procedure with its experimental component in the context of a life-threatening condition and with full informed consent from the patient and donor and immediate family. The major hazards (GVHD, infection, graft rejection, and leukemic relapse) have already been mentioned. Some poorly understood post transplant complications can lead to multiorgan failure including: inflammatory states such as a cytokine release syndrome and the endothelial damage syndromes (transplant microangiopathy, posterior reversible encephalopathy syndrome, diffuse alveolar hemorrhage, idiopathic pneumonitis, sinusoidal occlusive syndrome/veno-occlusive disease and delayed cardiovascular disease). Inevitably after transplantation, blood counts will decrease with an associated requirement for red blood cell and platelet transfusions, as well as a need for empiric antimicrobial use to prevent infectious complications. At the time of count recovery, there is also a small risk of pulmonary engraftment syndrome, manifested by non-infectious fever, pulmonary infiltrates, and dyspnea, which can be treated effectively with prompt administration of corticosteroids. The major discomforts during the post-transplant period are those of nausea, mucositis, anorexia, diarrhea, fever, malaise, and intolerance of the isolation period. Possible organ toxicities related to the high-dose preparative regimen include hepatic veno-occlusive disease and interstitial pneumonitis, which can be fatal.

The procedure will render the patient infertile. Because of this, we will discuss the possibility of sperm banking or egg retrieval with the subject before undergoing the transplant. There is no effect of the treatment on male hormone production or potency. Women stop ovulating and menstruating after the treatment, which also reduces estrogen production to low levels, inducing premature menopause. This is treated by hormone replacement therapy, which is important in order to conserve the body's store of calcium, amongst other reasons. In children before puberty, the treatment produces infertility, but with a small possibility of delayed recovery (about 10 years later). Puberty may be delayed, but often progresses normally. Although the whole transplant procedure may be accompanied by a stunting of growth, normal growth is subsequently resumed in the majority of children. In only a few cases, is it necessary to give growth hormone, testosterone (male hormone) or estrogen (female hormone) to complete normal growth and sexual development.

Allogeneic SCT is increasingly being offered to older individuals (> age 70) with the realization that chronological age alone is not as important as comorbidities, performance status or disease risk. Careful selection of patients with advanced age will mitigate the risk of complications. Nevertheless, older individuals will need to be carefully counseled prior to transplant regarding higher risks from transplant related mortality.

With amendment in April 2017, the protocol will no longer exclude subjects with prior allogeneic transplantation. In order to minimize risk and maximize benefit, candidates with a prior transplant will be carefully selected; they will be excluded if their prior transplant involved fully myeloablative conditioning or if their malignant disorder is highly refractory and deemed unlikely to benefit from an allotransplant approach. Analysis of patients on this protocol is standardized by disease risk index and comorbidity index, that will allow merging of the data of subjects with and without prior transplantation.

Related to the apheresis procedure:

The apheresis procedures will be performed in accordance with standard apheresis donation policies and procedures operative in DTM and will be in compliance with the Blood Donor Standards of the American Association of Blood Banks and the rules and regulations of the Food and Drug Administration. Adverse reactions to apheresis procedures are rare, but include:

- Pain and hematoma at the needle placement site
- Vasovagal episodes, characterized by transient hypotension, dizziness, nausea and rarely, syncope are seen in less than 2% of the procedures. Hypotension secondary to volume depletion is known for the rare potential for a cerebrovascular or cardiovascular event.
- Cutaneous or circumoral parasthesias, chills, nausea, heartburn and rarely muscle spasms may result from the use of citrate anticoagulant used to prevent clotting in the extracorporeal circuit. Citrate reactions are usually relieved by slowing the rate of the anticoagulant infusion and by administering oral calcium carbonate tablets or with intravenous calcium gluconate.

Prior to each apheresis, the potential risks associated with the procedure will be explained to the patient and a separate informed consent obtained.

Related to the CliniMACS CD34 Reagent Systems:

Theoretical risks to the patient could include system failure, user error, or patient reaction to selected product components (i.e. residual amounts of unbound CliniMACS CD 34 Reagent, Iron dextran, or human anti-mouse antibodies). For full text see Investigator's brochure for Miltenyi CliniMACS CD34+ system, Version 6, dated 5/27/2008, section 6, Summary and Guidance for the Investigator).

Related to the common transplant related drugs and radiotherapy.

Cyclosporine: CSA is metabolized primarily in liver but the major toxicity is renal. Side effects include renal impairment, reversible renal insufficiency, hemolytic uremic syndrome, elevated bilirubin and transaminases that normalize with continued administration or reduced dose, hypertrichosis, headaches, nausea, gingival hypertrophy, parasthesias (painful hands and feet), hypertension, hypomagnesium, bilirubinemia, hypertrichosis, nausea, tremor, and seizure. An extremely rare complication of cyclosporine is blindness, which may be irreversible. Posterior Reversible Encephalopathy Syndrome (PRES) is an increasingly recognized neurologic disorder seen in 1% of subjects on cyclosporine which manifest with acute to subacute hypertension and/or seizures. In the event of hypertension, subjects will be prescribed 1 or more medications to control blood pressure in an effort to decrease the risk of this complication.

Cyclophosphamide: immediate: tingling and metallic taste, nausea and vomiting, ADH-like effect, cardiotoxic at high doses (>70 mg/kg). Rare - pulmonary toxicity, urticaria and flushing, mucositis. Delayed: marrow suppression, nausea, mucositis, rash, hemorrhagic cystitis, myocardial damage, alopecia, infertility.

Fludarabine*:* myelosuppression, fever and chills, nausea and vomiting, malaise, fatigue, anorexia, weakness, and rarely hemolysis and pulmonary toxicity, hemolytic anemia and interstitial pneumonitis. Serious opportunistic infections have occurred in CLL subjects treated with fludarabine.

Methotrexate (used in the event of technical failure of graft processing resulting in CD3+ cells>1 x 10^6/kg): mucositis, nausea, dizziness, neutropenia, thrombocytopenia, malaise, fatigue, fever, melena, headaches, blurred vision, rashes, alopecia, elevated liver functions,
Hematologic: myelosupression (leukopenia [nadir 7 days] thrombocytopenia, anemia)
Hepatic: acute (elevated transaminases) and chronic (fibrosis and cirrhosis) hepatic toxicity. Chronic toxicity has generally occurred after prolonged use (generally 2 years or more) and after a total dose of at least 1.5 grams.

Urogenital: severe nephropathy or renal failure, azotemia, cystitis, hematuria; defective oogenesis or spermatogenesis, transient oligospermia, menstrual dysfunction and vaginal discharge; infertility, abortion, fetal defects. With high doses of methotrexate, close attention to renal function including adequate hydration, and urine alkanization are essential for safe administration.

Gastrointestinal: gingivitis, pharyngitis, stomatitis, anorexia, nausea, vomiting, diarrhea, hematemesis, melena, gastrointestinal ulceration and bleeding, enteritis. Should be used with extreme caution in the presence of peptic ulcer disease or ulcerative colitis. Therapy may be discontinued if ulcerative stomatitis or other severe GI adverse reactions occur.

Pulmonary: interstitial pneumonitis deaths have been reported, and chronic interstitial obstructive pulmonary disease has occasionally occurred. Pulmonary symptoms or a nonspecific pneumonitis may be indicative of a potentially dangerous lesion and require interruption of treatment and careful investigation; infection needs to be excluded. This lesion can occur at all dosages.

Skin: erythematous rashes, pruritis, urticaria, photosensistivty, pigmentary changes, alopecia, ecchymosis, telangiectasia, acne, furunculosis.

Central Nervous System: headaches, drowsiness, blurred vision. There have been reports of leukoencephalopathy following intravenous administration of methotrexate to subjects who have craniospinal irradiation. Aphasia, hemiparesis, paresis, and convulsions have also occurred following administration of methotrexate. Following low doses, occasional subjects reported transient subtle cognitive dysfunction, mood alteration, or unusual cranial sensations.

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

Related to antimicrobials in general*:* Allergic reactions, renal impairment (gentamicin, vancomycin, amphotericin, acyclovir), "red man" syndrome (vancomycin), hepatic damage (acyclovir, rifampin), nausea, anorexia, abdominal discomfort, pancytopenia, neutropenia, peripheral neuropathy, renal damage (Ganciclovir) (reversible on stopping drug).

Related to the total body radiation*:* side effects of radiation have been well described. The most common include nausea and mucositis. Mucositis is associated with bacteremias and sepsis. There also exists a risk of hypothyroidism, cataracts, interstitial pneumonitis, nephropathy, and an unspecified long term risk of developing secondary malignancies. Importantly, the majority of the nonneoplastic effects were sub clinical and/or reversible.

13.3.2 The Donor

Related to filgrastim (G-CSF). The hazard to the donor is low. The discomfort from G-CSF mobilization and leukapheresis for collection of blood stem cells are probably lower than those associated with marrow harvesting. G-CSF has been given to large numbers of normal donors without major side effects or long-term consequences. The immediate side effects of G-CSF are bone pain, fatigue, insomnia, myalgia and headache. These are usually mild and are self-limiting. Reversible thrombocytopenia, with platelet counts falling to the region of 100,000 /cu mm is frequent. Although regarded as safe and well tolerated, G-CSF administration could result in splenic rupture, a potentially life-threatening complication. Up to now five cases of splenic rupture have been reported in healthy donors (Becker et al. 1997, Falzetti et al. 1999, Balaguer et al. 2004, Dincer et al. 2004, Nuamah et al. 2006). Donors will be asked to avoid vigorous activities and to report any left upper abdominal and/or shoulder pain to the research team or the on-call physician for the NIH Department of Transfusion Medicine at 301-496-1211.

Subjects with ongoing ischemic heart disease have been reported to have angina seemingly temporally-related to G-CSF administration and apheresis (Kopp*, et al* 2007, Vij*, et al* 1999). In addition, a rare occurrence of pulmonary hemorrhage has been reported in a healthy donor who was a cigarette smoker and had underlying pulmonary disease (Kopp*, et al* 2007). Finally, donors with sickle cell trait have been reported to develop vaso-occlusive crises when treated with G-CSF, necessitating special precautions (Kang*, et al* 2002).

Related to central line placement. It is estimated that about 50% of the donors will require intravenous line placement to successfully complete apheresis. Intravenous line placement in the central vein using an apheresis catheter carries a small risk of bleeding, bruising or pain and a very low risk of accidental injury to the adjacent artery and nerve. Central line placement in the upper circulation carries a small risk of puncture of a pneumothorax. Some subjects may experience a vasovagal reaction (lightheadedness, or, rarely, fainting due to temporary lowering of blood pressure).Using only experienced staff for the procedure minimizes these risks.

In exceptional instances the donor does not mobilize well and is required to receive plerixafor or to donate PBPC a second time. Plerixafor is an FDA approved mobilizing agent and would have a standard indication in this setting. The risks of plerixafor are mainly GI upset. There is no additional risk to the donor from a second PBPC donation.

***Related to Apheresis*:** see section 13.3.1.

13.4 Research Risks in Relation to Research Benefits

13.4.1 For Adult Transplant Subjects

Allogeneic stem cell transplantation is the treatment of choice for many subjects with leukemia and other hematologic malignancies, however it entails serious discomforts and hazards for the patient (section 13.3). -The investigational component of this transplant protocol aims to decrease non-relapse mortality and improve overall survival by reducing GVHD and the exposure to prolonged immunosuppression. This approach is theoretically likely to increase the risk of infections, relapse/progression and slow donor lymphoid engraftment

Clinically, this approach is ethically acceptable because we will target a patient group with lethal hematologic malignancies and no other curative treatment options who have made an informed decision to opt for a procedure which they understand offers a chance of prolonged disease-free survival but with a risk of death from treatment failure. Implicit in this decision is the opportunity to weigh all treatment options after full information has been given to them by the NIH team and the referring physician.

Therefore for adult transplant recipients on this protocol, the research involves greater than minimal risk to subjects with the prospect of direct benefit (45 CFR 46.102).

Exclusion of pregnant women: The exclusion of pregnant women is justified on medical grounds as pregnancy is an absolute contraindication for myeloablative doses of chemo-radiation.

13.4.2 For Pediatric Transplant Subjects

The investigational component of this transplant protocol aims to decrease non-relapse mortality and improve overall survival by reducing GVHD and the exposure to prolonged

immunosuppression. This approach is theoretically likely to increase the risk of infections, relapse/progression and slow donor lymphoid engraftment. The inclusion of children satisfies the criteria set forth in 45 Code of Federal Regulations 46, Subpart D: 46.405 as follows:

(a) *the risk is justified by the anticipated benefit to the subjects*: We are offering pediatric subjects with a probably lethal hematological disease, incurable with conventional treatments other than allogeneic stem cell transplantation, an alternative to symptomatic therapy.

(b) *the relation of the anticipated benefit to the risk is at least as favorable to the subjects as that presented by available alternative approaches*. The protocol aims to decrease the risk of transplant-related mortality, thus making more subjects candidates for potentially curative therapy and

(c) adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians, as set forth in 46.408.

Therefore for pediatric transplant recipients on this protocol, the research involves greater than minimal risk but presents the prospect of direct benefit to the individual subjects (45 CFR 46.405).

13.4.3 For Adult Donors:

An HLA 6/6 identical family member will be co-enrolled into this study as a stem cell donor. The stem cell collection aspect of this protocol is not investigational. Despite the risks associated with this standard procedure (13.3.2), potential benefit does exist for family donors. The donor derives psychosocial benefit from donating stem cells both at the time of donation and possibly into the future, especially in view of the reduced life expectancy due to this disease in a family member. Other potential benefits include detection of illnesses, determination of blood cell counts, and evaluation of kidney and liver function in the potential donor at the time of screening. Therefore, participation as a stem cell donor on this protocol involves minimal risk to subjects. For those who choose to participate in the Laboratory Research Studies involving bone marrow sampling, the risk is greater than minimal but presents the prospect for direct benefit(45 CFR 46.102).

13.4.4 For Pediatric Donors:

Stem cell transplant is an accepted standard clinical intervention for the diseases under investigation. The donor would be donating stem cells and lymphocytes collected separately during two apheresis procedures to his/her family member regardless of the objectives of this research protocol. The stem cell and lymphocyte collection procedures are not considered part of the research for the donors and the risks of the stem cell and lymphocyte collection procedures would not be considered risks of the research for the pediatric donors. Therefore, participation as a stem cell donor on this protocol is considered exempt from the criteria set forth in 45 CFR 46, Subpart D.

13.4.5 For Pediatric Donors – Healthy Volunteers- Involved in Laboratory Research Studies.

The inclusion of children satisfies the criteria set forth in 45 Code of Federal Regulations 46, Subpart D: 46.404 as follows:

(1) *The research does not involve greater than minimal risk*. There is no added risk associated with the amount and method of collection for the additional blood for research

obtained concurrently with clinically indicated sampling. We are not collecting bone marrow aspirate for the lab research studies.

Research specimens will be stored in the PI's laboratory. Samples will be assigned a unique code known only to the PI, which will serve as a link to the child's name and clinical information collected as part of this research protocol. No samples will be provided to investigators outside the branch without permission of the IRB, therefore confidentiality will be protected.
Only those laboratory tests approved by the IRB and involving not greater than minimal risk to subjects will be conducted (See Appendix A). Research will not include genetic testing, thus there is no risk related to genetic testing.

(2) adequate provisions are made for soliciting the assent of the children and permission of their parents or guardians, as set forth in 46.408.

Therefore, participation of pediatric donors in laboratory research on this protocol does not involve greater than minimal risk (45 CFR 46.404).

13.5 Informed Consent Processes and Procedures

Adults: The investigational nature and research objectives of this trial, the procedure and its attendant risks and discomforts will be carefully explained to the subject and a signed informed consent document will be obtained prior to entry onto this study. Drs. Barrett or Battiwalla or other senior physicians who are specifically listed as associate investigators capable of obtaining consent on the front page of the protocol will lead this discussion.

If the patient is a minor, a minor assent will be sought. Where deemed appropriate by the clinician and the child's parent or guardian, the child will also be included in all discussions about the trial and a minor's assent will be obtained. The parent who signs the consent for the minor must be a parent or legal guardian. The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent. When the assent is not age appropriate, the study will be explained to the child and the assent will be obtained verbally from the child. In cases where parents share joint legal custody in making medical decisions of their child (e.g. by a custody agreement or court order) both parents must give their parental permissions regardless of level of risk of the research. Exceptions may be made if one parent is deceased, becomes incompetent or is not reasonably available (e.g. in prison).

If the donor is a minor, assent will not be sought until an evaluation by a social worker and mental health specialist (psychologist or psychiatrist) is completed to determine the minor donor's willingness to participate. As detailed above, the parent who signs the consent for the minor must be a parent or legal guardian. Where deemed appropriate by the clinician, and the child's parent or guardian, the child will also be included in all discussions about the trial and a minor donor's assent will be obtained. The investigators are requesting a waiver from the IRB to allow only one parent to sign the informed consent to enter a child on the protocol. The parent or guardian will sign on the designated line on the informed consent attesting to the fact that the child had given assent. In cases where parents share joint legal custody in making medical decisions of their child (e.g. by a custody agreement or court order) both parents must give their parental permissions regardless of level of risk of the research. Exceptions may be made if one parent is deceased, becomes incompetent or is not reasonably available (e.g. in prison).

If a child (donor or transplant recipient) dissents, the IRB and Bio-ethics will be consulted.

If the minor subject is a female of childbearing age, she will be informed about pregnancy testing and will be told that if her pregnancy test is positive, we will counsel her and help her tell her parents or we will tell her parents with her permission. Is she does not agree she will be advised not to sign the assent.

Decisionally impaired: The IRB and Bio-ethics Department will be consulted should there be a decisionally impaired patient (donor or recipient) identified as a potential candidate for enrollment in this protocol.

At any time during participation in the protocol that new information becomes available relating to risks, adverse events, or toxicities, this information will be provided orally or in writing to all enrolled or prospective patient participants. Documentation will be provided to the IRB and if necessary, the informed consent will be amended to reflect relevant information.

Re-Consent for Minors when they reach the age of majority: When a pediatric subject reaches age 18, continued participation will require re-consenting of the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained. Should sample or data analysis continue following completion of active participation and the subject has reached 18 years of age, we will attempt to contact the subject using the last known contact information to obtain consent for continued use of data or samples collected during their prior visit. Given the length of time that may have transpired for some of the subjects since their last visit for this study, we request waiver of informed consent for those individuals who after good faith efforts to contact them, we are unable to contact.

Requirements for Waiver of Consent consistent with 45 CFR 46.116 (d), each of which must be addressed in relation to the protocol:

(1) The research involves no more than minimal risk to the subjects.
    a. Analysis of samples and data from this study involves no additional risks to subjects.
(2) The waiver or alteration will not adversely affect the rights and welfare of the subjects.
    a. This is an FDA-regulated study and as such, we are mandated to retain all samples, once collected, regardless of the age of the subject at the time of collection. Retention of these samples or data does not affect the welfare of subjects.
(3) The research could not practicably be carried out without the waiver or alteration.
    a. Considering the length of time between a minor's enrollment and their age of majority, it is possible that more than a few subjects may be lost to follow up. A significant reduction in the number of samples analyzed could impact the quality of the research.
(4) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.
    a. We only plan to request a waiver of reconsent for those subjects who have been lost to follow-up.

Non-English speaking subjects: If there is an unexpected candidate for enrollment of a research participant for which there is no translated extant IRB approved consent document, the principal investigator and or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, 45 CFR 46.117 (b) (2), and 21 CFR50.27 (b) (a) 0. The summary that will be used is the English version of the extant IRB approved consent document.

We request prospective IRB approval of the use of the short form for up to a maximum of **5** research participants in a given language and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form. Should we reach the threshold of 5, we will notify the IRB of the need for an additional use of the Short Form and we will have the consent document translated into the given inherent language.

13.6 Conflict of Interest and Tech Transfer Agreements

The Principal Investigator assured that each associate investigator listed on the protocol title page received a copy of the NIH's Guide to preventing conflict of interest. Investigators added subsequent to the initial circulation will be provided a copy of the document when they were added. Copies of the Conflict of Interest Statement were forwarded to the Clinical Director. No initial members of the research team reported a potential conflict of interest.

This protocol has no associated patents or CRADAs. The protocol has the following associated CTA:

- between Miltenyi Biotec Incorporated and the National Heart, Lung and Blood Institute, entered on Nov 15, 2006 by which Miltenyi agrees to make available its CliniMACS CD34 reagent cell selection system for clinical research purposes.

This protocol has the following MTAs that is partially executed:

- Between NHLBI and Catherine Bollard, MD, Children's National Medical Center, 111 Michigan Ave, NW, Washington DC, 20010, as part of a collaboration to evaluate NK cell phenotype and function and to determine whether the function can be improved by ex vivo expansion.

- Between NHLBI and Pawel Muranski, MD, Columbia University Medical Center**,** 630 West 168th Street, New York, NY 10032. The de-identified coded samples from healthy donors and healthy volunteers enrolled in 13-H-0144 study may be sent to Dr. Muranski to be used in development of future clinical trials.

13.7 FWA Coverage Agreement

Dr. Muranski has recently assumed the role of Director of Cellular Immunotherapy at Columbia University Medical Center, NY and will be analyzing identifiable data as an Associated Investigator in this protocol. Dr. Muranski's role in the research will be limited to data analysis. An FWA coverage agreement to cover this activity will be executed by Dr. Muranski and Dr. Ito, once this amendment is approved.

14.0 PHARMACEUTICALS

14.1 CliniMACSCD34 Reagent System (see Investigator's Brochure, Version 6, dated 5/27/2008)

14.1.1 Investigational Product: the CliniMACS® CD34 Reagent System is a medical device that is used *in vitro* to select and enrich specific cell populations. When using the CD34 Reagent, the system selects CD34+ cells from heterogeneous hematological cell populations for transplantation in cases where this is clinically indicated. The CliniMACS CD34 Reagent System is comprised of four primary component**s:**

- CliniMACS CD34 Reagent: a sterile monoclonal antibody reagent specific for CD34+ cells
- CliniMACS plus Instrument: a software controlled instrument that processes the blood sample (cell product)
- CliniMACS Tubing Sets: single-use, sterile, disposable tubing sets with two proprietary cell selection columns (CliniMACS Tubing Set and CliniMACS Tubing Set LS)
- CliniMACS PBS/EDTA Buffer: a sterile, isotonic phosphate-buffered, 1 mM EDTA, saline solution, used as external wash and transport fluid for the *in vitro* preparation of blood cells.

14.1.2 Physical, Chemical and Toxicological Information

- CD34 Reagent Description: the CliniMACS CD34 Reagent is a dark amber, non-viscous, colloidal solution containing the antibody conjugate in buffer. The conjugate consists of a monoclonal antibody towards the class II epitope of the human CD34 antigen. The murine monoclonal IgG1 antibody is covalently linked to dextran beads that have an iron oxide/hydroxide core and are super paramagnetic.

- Safety testing of the CD34 Monoclonal Antibody: cell banking, cell culture, as well as subsequent purification of the antibody, follow the applicable current international guidelines as described in Section 4. The testing of the CD34 Master Cell Bank, the End of Production Cells, the CD34 mAb pooled cell culture harvest (unprocessed bulk) and the purified CD34 monoclonal antibody (mAb) have been completed and the purified CD34 mAb has been released for manufacturing of the CD34 Reagent. Additionally, the viral inactivation/removal steps used in the purification of the CD34 monoclonal antibody have been validated.

- Safety testing of the CD34 Reagent: detailed toxicity studies have been undertaken to assess the safety of the CD34 Reagent when delivered in dosages significantly greater than the projected maximum dosage anticipated in clinical use. The testing was performed in accordance with 21 CFR §58, Good Laboratory Practices for Nonclinical Laboratory Studies. A summary of this testing is provided in **Table 6**.

Table 6: Toxicity studies to assess the safety of the CD34 Reagent

Summary of Toxicology of the CliniMACS CD34 Reagent	
Test	Results
Human Cryosection Cross Reactivity Study	CD34 Reagent specifically reacted with cell types known to possess the CD34 antigen. Not considered toxicologically significant.
Interspecies Cross Reactivity Study	CD34 antibody does not cross react with non–human primate hematopoietic cells expressing the CD34 antigen. These species could be used for safety testing.
Subchronic Toxicity	No Toxicity
Cardiovascular Safety Study in Rhesus Monkeys	No drug-related effects on mean arterial pressure, mean right ventricle pressure, cardiac output, ECGs, respiration rate, heart rate or cage side observations were noted when escalating doses of CD34 Reagent
Irritation	No Irritation
Hemocompatibility	Compatible with human blood
Sterility assay of final container	Reproducibly sterile product

14.1.3 Safety Testing of CliniMACS® System Components (Instrument, Tubing Sets and PBS/EDTA Buffer) Biocompatibility Testing of the CliniMACS System components (Tubing Sets and PBS/EDTA Buffer) was performed according to ISO 10993. The requirements of ISO 10993 were fulfilled for the CliniMACS CD34 Reagent System. The CliniMACS plus Instrument has

been tested for electrical safety and the potential for fire, shock, explosion, or mechanical damage. Potential safety issues have been reduced by using a design to meet European standards EN 60601-1. It is UL and CSA listed and approved.

14.1.4 Overall Safety of the CliniMACS® CD34 Reagent System: The results summarized in this Investigator's Brochure support that CliniMACS CD34 Reagent System is sufficiently safe for clinical use with human subjects. The potential application of the CliniMACS CD34 Reagent is broad. Infusion of purified CD34+ cells is indicated in a number of clinical applications after myeloablative or lymphoablative therapy including reduction of tumor cells in the transplant and depletion of T cells for autologous (autoimmune diseases) and allogeneic transplantations.

Individual risk analysis on the therapeutically used target cells isolated in conjunction with CliniMACS CD34 Reagent System should be addressed by each site using these cells. A European safety study, ACS 950101, for the CliniMACS System was published in Bone Marrow Transplantation 25; 243-49, February 2001. The study was designed to meet European Essential Requirements 3 and 14 (MDD 93/42/EEC) and was conducted per EN540 to support the CE Marking of the device (received December 1997).

The initial clinical study with the CliniMACS System was conducted in subjects undergoing high-dose chemotherapy for breast cancer. The purpose of the European Safety Study was to show:

- Suitability of the CD34 Reagent and other CliniMACS components for selection of CD34+ cells with high, yield, purity viability and safety
- CD34+ cells can safely be administered to subjects after myeloablative chemotherapy
- Selected CD34+ cells are effective in reconstituting the hematopoietic system after myeloablative chemotherapy
- Rate of device failures

Cells were isolated from leukapheresis products from sixty-five subjects enrolled in the study. Fifty-four subjects received selected CD34+ cells and fifty-two were evaluable for engraftment as summarized in the table below (one patient died 5 days post-transplant and prior to engraftment and one patient did not recover platelet counts even after back-up cells were infused). All subjects receiving selected cells completed 60 and 100-day follow-up after infusion, during which time their hematological and immune status were monitored, as was HAMA production. A summary of the results of the European Clinical Trial is provided below.

Table 7: European Clinical Trial results

Time to Hematological Engraftment After Infusion of CliniMACS Selected CD34+ Cells		
Time to Engraftment (Days)		
	Platelets (≥ 20 x 109/L)	Neutrophils (≥ 500/uL)
Median (Kaplan Meier)	11.6	9.1
SD	6.05	5.81
Quartile range (Kaplan Meier)	10.0-12.0	8.0-10.0
Range	8-29	8-11
Number	52	52

The following conclusions were made regarding this clinical study:

- The CliniMACS CD34 Reagent System selects CD34+ cells from heterogeneous hematological cell populations. The resulting CD34+ product is of high purity (median of 96.1 %, range 27.4

– 99.4 %); the median recovery of CD34+ cells was 52.3 % (range 15.2 – 146.3%). The reported performance results are similar to those seen in pre-clinical studies.

- Infusion of selected, autologous CD34+ cells after high dose chemotherapy resulted in rapid engraftment (see Table 2). These data are comparable to previously reported results, using bone marrow or peripheral blood cells as stem cell source. Following cyclophosphamide, thiotepa and carboplatin (CTCb), Weaver et al. reported median times to platelet and neutrophil engraftment of 9 days (range 0-53 days) and 9 days (range 5-26 days), respectively. Also after CTCb, Elias et al., reported median times to platelet and neutrophil recovery of 12 days (range 8-134 days) and 14 days (range 10-57 days), respectively (Elias*, et al* 1992, Weaver*, et al* 1995).
- The selection process has no discernable effect on cell viability or sterility.
- There were no adverse events or device malfunctions reported as related to the infusion of the cells or use of the CliniMACS CD34 Reagent System. None of the subjects were reactive for HAMA post infusion. There were no reports of late engraftment failure or evidence of delayed immune reconstitution.

14.2 CYCLOPHOSPHAMIDE (Cytoxan, Neosar)

Supply: Commercially available.
Product description: Cyclophosphamide is available as a lyophilized powder for injection in multiple vial sizes.
Preparation: Cyclophosphamide powder for injection should be reconstituted with sterile water for injection to yield a concentration of 20 mg/mL as described in the product labeling. Once reconstituted, the prescribed dose will be further diluted in 250 mL of 0.9% sodium chloride injection or 5% dextrose in water for intravenous administration over 60 minutes.
Storage and stability: vials of cyclophosphamide are stored at room temperature. Once reconstituted as directed, solutions of cyclophosphamide are stable for 24 hours at room temperature, or 6 days when refrigerated at 2-8^{o} C.
Route of administration: the prescribed dose of cyclophosphamide will be diluted in an additional 250 mL of 0.9% sodium chloride injection or 5% dextrose in water for intravenous administration over 60 minutes.
Toxicities: see section 13.3.1.

14.3 CYCLOSPORINE (Gengraf, Sandimmune, Neoral)

Supply / product description: cyclosporine will be obtained by the NIH Clinical Center Pharmacy Department from commercial sources and is available in capsules (25 mg and 100 mg), USP [MODIFIED], oral solution (100 mg/ml), USP [MODIFIED], and as a parenteral concentrate for injection (50 mg/ml). When oral capsules are prescribed for this protocol, the cyclosporine capsules, USP [NON-MODIFIED] should NOT be used.
Preparation: for parenteral doses, each milliliter of concentrate (50mg/ml) should be diluted in 20 to 100ml of dextrose 5% in water or sodium chloride 0.9%. Parenteral doses of cyclosporine will be prepared in non-PVC containers and infused with non-PVC administration sets/tubing. Oral cyclosporine solution may be mixed in orange juice or other beverages, but not milk.
Storage and stability: capsules, oral solution, and ampules of parenteral concentrate bear expiration dates and are stored at room temperature and protected from light. Cyclosporine concentrate for injection that has been diluted to a final concentration of approximately 2mg/ml is stable for 24 hours in 5% dextrose or 0.9% sodium chloride injection in glass, PVC or non-PVC plastic containers. To minimize the potential for absorption to PVC plastic bags and tubing as well the leaching of phthalate plasticizer (DEHP) into the solution, only non-PVC plastic bags and intravenous administration sets should be utilized.
Administration: cyclosporine may be given intravenously or orally.
Toxicities: refer to section 13.3.1

14.4 FLUDARABINE PHOSPHATE (Fludara)

Supply: commercially available.

Product description: fludarabine phosphate is commercially available as both a lyophilized powder for injection in vials containing 50 mg of fludarabine phosphate with mannitol 50mg and sodium hydroxide for pH adjustment and a solution for injection in 2 mL vials containing 50 mg of fludarabine phosphate (25 mg/mL of fludarabine) with 25 mg/mL mannitol and sodium hydroxide for pH adjustment.

Preparation: fludarabine lyophilized powder for injection should be reconstituted with 2 mL of sterile water for injection, USP to a concentration of 25 mg/mL. The prescribed dose of fludarabine should be diluted in 100 mL of either 0.9% sodium chloride or 5% dextrose in water for intravenous administration over 30 minutes.

Storage and stability: fludarabine vials should be stored under refrigeration between 2°-8° Celsius (36°- 46° F). Reconstituted fludarabine phosphate is chemically and physically stable for 24 hours at room temperature or for 48 hours if refrigerated. The manufacturer recommends use of either the reconstituted powder for injection or the solution for injection (once diluted for administration) within 8 hours because neither product contains an antimicrobial preservative.

Administration: the prescribed dose of fludarabine should be diluted in 100 mL of either 0.9% sodium chloride or 5% dextrose in water for intravenous administration over 30 minutes.

14.5 FILGRASTIM (G-CSF, Neupogen)

Supply: commercially available.

Product description: filgrastim injection is available in a concentration of 300mcg/ml in 1ml (300mcg) and 1.6ml (480mcg) vials.

Preparation: for subcutaneous administration, the appropriate prescribed dose is drawn up from the vial with no further dilution prior to administration. For intravenous administration, the commercial solution for injection should be diluted prior to administration. It is recommended that the prescribed dose be diluted with dextrose 5% in water (DO NOT DILUTE WITH NORMAL SALINE) to a concentration greater than 5mcg/ml. Filgrastim diluted to concentrations between 5 and 15mcg/ml should be protected from absorption to plastic materials by the addition of Albumin (Human) to a final concentration of 2mg/ml. When diluted in 5% dextrose or 5% dextrose plus Albumin (Human), filgrastim is compatible with glass bottles, PVC and polyolefin IV bags, and polypropylene syringes.

Storage and stability: filgrastim for injection should be stored in the refrigerator at 2° to 8°C (36° to 46°F). Avoid shaking.

Route of administration: subcutaneous injection or intravenous infusion over 15-30 minutes.

14.6 METHOTREXATE

Supply: Commercially available

Product Description: Methotrexate is available as a 25 mg/mL preservative-free isotonic solution for injection.

Preparation: The desired does will be diluted in 25 to 50 ML of 5% dextrose in water of 0.9% sodium chloride.

Storage and Stability: Methotrexate should be stored at room temperature and protected from light. Once the prepared does is diluted for administration, the solution is stable for 24 hours refrigerated or at room temperature when protected from light.

Route of Administration: Methotrexate will be given as an IV infusion over 15 minutes.

Toxicities: refer to section 13.3.1

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16.0 APPENDICES

APPENDIX A NHLBI HEMATOLOGY BRANCH LABORATORY RESEARCH STUDIES -2/5/2013

	DESCRIPTION OF LABORATORY STUDY BY BRANCH SECTION	Does this test pose a greater than minimal risk to pediatric subjects per 45 CFR 46.404?	Does this test pose a greater than minimal risk to healthy pediatric donors per 45 CFR 46.404?
A	**Stem Cell Allotransplantation Section (Dr. A. John Barrett)**		
A.1	Measurement of lymphocyte function and immune responses directed toward allogeneic tissues, malignant cells, and infectious agents. Assay of a variety of antigens, including standard proliferation, cytotoxicity, and intracellular cytokine detection including GVHD predictive markers. Measurement of antigen-specific responses including employment of tetramers, ELISPOT technique, gene amplification-based assays, and flow cytometry. Selection of cells using immunomagnetic beads or flow cytometry. Culture, expansion, and selection of cells. Surface marker analysis of PB MC using flow cytometry. Cytokine/chemokine analysis of plasma/serum samples using ELISA and/or Luminex techniques.	No	No
A.2	Generation of cell lines for the study of immune cell interactions with other cells. Transformation of B-lymphocytes using Epstein-Barr virus. Derivation of malignant cell lines from patient leukemic or solid tumor samples.	No	No
A.3	Infection of cells and cell lines with recombinant genes to ascertain the effects of expressed molecules on immune responses and on growth and development. Transfection of cell lines with specific molecules to study antigen-specific responses.	No	No
A.4	Assays of peripheral blood and bone marrow progenitor cells including primitive and late erythroid progenitor-derived colonies, myelomonocytic colonies, and primitive multi- potential progenitor-derived colonies.	No	No
A.5	Injection of human cells into experimental animals to study the immune system and the growth of normal and malignant cells under varying conditions.	No	No
A.6	Testing of selection methods, cell isolation, and cell expansion leading to the development of new cell-based therapies requiring scale-up for clinical application.	No	No
A.7	Identification of individual T cell clones by their T cell receptor sequence.	No	No
A.8	Measurement of tumor and tissue specific antigens in cells of subjects and donors by mRNA,protein, or peptide expression in cells or fluids.	No	No
A.9	Laser capture micro dissection of cells from biopsies for GVHD to determine clonotypes.	No	No
A.10	DNA and RNA typing of genes that control immune responses in lymphocytes.	No	No
A.11	Microassay studies utilizing cellular DNA, cDNA, and RNA for neoplasia and host-tumor interactions.	No	No
A12	Serum, blood and tissue markers of post-transplant tissue injury, particularly endothelial cell damage. Investigation of transplant survivors for metabolic derangements related to cardiac and vascular risk such as lipoprotein profiles, insulin	No	No

	resistance, diabetes markers, growth hormone signaling, hypertension, renal dysfunction.		
B	**Molecular Hematopoiesis Section (Dr. Cynthia Dunbar)**		
B.1	Flow cytometric analysis of cell surface and cytoplasmic proteins, including cell adhesion molecules, putative retroviral receptors, and markers of differentiation, using bone marrow and mobilized peripheral blood cells.	No	No
B.2	Hematopoietic progenitor-derived colony ascertainment in vitro (as described above), and engraftment of immunodeficient mice for detection of human stem cell number and function.	No	No
B.3	Testing ability of hematopoietic progenitor cells to be transduced with retroviral, lentiviral, and novel gene transfer vectors in vitro.	No	No
B.4	Reprogramming of adult mature cells, including skin fibroblasts and blood cells, into induced pluripotent stem cells in vitro.	No	No
C	**Cell Biology Section (Dr. Neal Young)**		
C.1	Studies of blood and bone marrow hematopoietic progenitor numbers, including early and late erythroid progenitors, myelomonocytic progenitors, and multi-potential progenitor cells. In addition, bone marrow may be placed in long-term bone marrow culture to assess the function of stroma and stem cells and to assay more primitive progenitors, as well as organelle culture. Whole or selected bone marrow populations are cultured short-term for CD34 cell expansion.	No	No
C.2	Assays of apoptosis in hematopoietic cells and their progeny, using flow cytometric methods such as annexin and caspase-3 staining, propidium iodide uptake, and mitochondrial permeability tests.	No	No
C.3	Separation and functional study of cell populations characteristic of paroxysmal nocturnal hemoglobinuria, identified by absence of glycosylphosphatidylinositol anchored proteins.	No	No
C.4	Studies of mutation rates in hematopoietic cells and in buccal mucosa cells, using conventional hypoxanthine phosphoribosyltransferase activity functional assays, sequencing of mitochondrial DNA after specific gene amplification, and measurement of GPI-anchored deficient cells in blood and bone marrow.	No	No
C.5	Assays of immune function of T-cells, including intracellular cytokine staining, ELISPOT, semiquantitative gene amplification for gamma-interferon, tumor necrosis factor, interleukin-2, and other cytokines, and functional assessment in co-culture using specific neutralizing monoclonal antibodies. In addition, peripheral blood lymphocytes are subjected to spectratyping for CDR3 size distribution as well as nucleotide sequence of CDR3 peaks obtained.	No	No
C.6	Studies of engraftment of human normal and diseased bone marrow and peripheral blood in immunodeficient mice in order to determine the presence of hematopoietic repopulating stem cells as well as functional differences among selected populations.	No	No
C.7	Flow cytometric analysis of blood and bone marrow for lymphocyte phenotype, especially for evidence of activation of lymphocytes, for markers of apoptosis, and for antigens associated with primitive and mature hematopoietic cell populations.	No	No
C.8	Flow cytometric analysis of blood and bone marrow for hematopoietic stem cell progenitors and CD34 positive cells.	No	No
C.9	Studies of chromosomal instability in myelopdysplastic syndromes including BM cell and CD34 cell response to PAS crosslinking and examination of the cytotoxic effect of lymphocytes to the abnormal clone of cells.	No	No
C.10	Surface Enhanced Laser/Desorption Ionization (SELDI) time-of-flight mass spectrometry (Ciphergen) (proteomics methodology).	No	No
C.11	Mitochondrial DNA (mtDNA) sequence heterogeneity.	No	No

C.12	Measurement of EBV viral load.	No	No
C.13	Measurement of EBV LMP-1 via RT-PCR for LMP-1 RNA or flow cytometry for LMP-1.	No	No
C.14	Outgrowth assay of EBV transformed B cells.	No	No
C.15	Quantification of serumchemokines and cytokines (e.g. SDF-1, IL-10, IL-6, CXCR4, CXCL12).	No	No
C.16	Quantification of EBV cytotoxic T cells (tetramerstaining).	No	No
C.17	Telomere length measurement by Southern blot, Q-PCR, flow-fish, in situ hybridization and STELA	No	No
C.18	Telomere repair complex gene mutations by nucleotide sequencing of some or all of the following: *DKC1* ,*TERC*, *TERT*, *SBDS*, *NOp10* , *NHP2*.	No	No
C.19	Analysis of inflammatory markers and/or bacterial, viral, fungal or protozoal elements in plasma or serum using molecular, colorimetric, enzymatic, flow cytometric or other assays in subjects receiving immunosuppressive therapy, chemotherapy and/or bone marrow transplantation.	No	No
C.20	Confocal microscopic imaging of bone marrow.	No	No
C.21	Characterization of intracellular signaling proteins by cell permeabilization and flow cytometry, and quantitative immunoblots.	No	No
C.22	Assays for chromosomal aneuploidy by florescence in situ hybridization (FISH) and other molecular techniques.	No	No
C.23	Conversion of human dermal fibroblasts into hematopoietic progenitors using Oct4 transfection.	No	No
C.24	Quantification of gene expression with RNA-seq	No	No
C.25	Characterization of chromatin and promoter/enhancer landscapes with ATAC-seq	No	No
C.26	Measurement of protein markers with SomaLogic's SOMAscan assay	No	No
D	**Virus Discovery Section (Dr. Neal Young) THESE ASSAYS WILL NOT BE PERFORMED ON SAMPLES FROM HEALTHY PEDIATRIC DONORS**		
D.1	Assays of serum, blood cells, and bone marrow cells for B19 parvovirus and possible B19 variants using gene amplification, cell culture, and hematopoietic colony inhibition assays.	No	N/A
D.2	Assays of blood, bone marrow, liver, and other tissues for potentially novel viruses, using a variety of techniques including RNA and DNA assays, differential display, gene amplification with conserved and random primers, cell culture assays, immunohistochemical methods, and inocculation of mice, rabbits, and monkeys, as well as antibody measurements.	No	N/A
D.3	Assays of blood, bone marrow, and liver for known viruses, including herpesviruses such as cytomegalovirus, human herpesviruses 6, 7, and 8, enteric viruses such as A-6, circiviruses, and parvoviruses, using assays as in (2).	No	N/A
D.4	Spectra-typing of blood cells to determine response to known or putative viral infections.	No	N/A
D.5	HLA typing or subtyping to determine risk factors/determinants for hepatitis-AA studies.	No	N/A
D.6	Cytotoxic lymphocyte assays with intracellular cytokine measurement for determining anti-viral response and lymphocyte cloning to obtain clones with specific antiviral activity.	No	N/A
E	**Solid Tumor Section (Dr. Richard Childs)**		
E.1	Cr51 cytotoxicity assay to evaluating killing of patient tumor cells by patient NK cell clones and T-cells.	No	No
E.2	ELISA for IL-12 maturity of DC's made from subjects monocytes.	No	No
E.3	ELISA for IFN ã to evaluate specificity of CTL clones.	No	No

E.4	H thymidine uptake to evaluate proliferation potential of antigen specific T-cells.	No	No
E.5	PCR of STR to assess chimerism status of cellular subsets grown in-vitro or retrieved from subjects post-transplant.	No	No
E.6	Flow sorting of PBL and/or tissue samples to evaluate chimerism of different subsets.	No	No
E.7	Surface marker analysis of peripheral blood mononuclear cells using flow cytometry.	No	No
E.8	cDNA expression arrays to evaluate T-cells expression/gene patterns in subjects with GVHD and a GVT effect.	No	No
E.9	Geno typing of tumor or tissue samples by high density cDNA arrays.	No	No
E.10	VHL mutation analysis on kidney cancer tissue.	No	No
E.11	Transduction of dendritic and tissue cells with tumor antigens using plasmids, viral vectors and hybrid fusions.	No	No
E.12	Lasar capture microdisection of cells from tumor biopsies and tissue samples to determine origin (donor vs patient).	No	No
E.13	Quantification of polyoma virus BK exposure by serology and PCR in stem cell transplant donors and recipients from blood and urine samples.	No	No
E.14	Quantification of polyoma virus BK specific T cells in stem cell transplant donors and recipients from peripheral blood samples.	No	No
E.15	Determination of origin of neovasculature endothelial cells in tumor and tissue samples obtained from subjects post transplant.	No	No
E.16	Quantification of lymphocyte subsets CD34 progenitors and endovasculator progenitors in G-CSF mobilized peripheral cell allografts.	No	No
E.17	Testing for polyoma virus BK latency in CD34 progenitors, B cells and T cells in the G-CSF mobilized peripheral cell allografts.	No	No
E.18	Determination of etiology of membraneous nephropathy using serum from subjects.	No	No
E.19	Serum Proteomic patterns analysis to diagnose complications related to allogeneic transplantation.	No	No
E.20	Determine cell origin (donor vs patient) of tissue samples using IHC, IF, sorting, and FISH.	No	No
F	**Lymphoid Malignancies Section (Dr. Adrian Wiestner)**		
F.1	Culture of cells from research subjects to investigate molecular disease mechanisms, model host tumor interactions, and to test effect of drugs on cell survival and cellular functions.	No	No
F.2	Generation of stable cell lines for the study of hematologic malignancies.	No	No
F.3	Modifications of cells using standard expression systems or biologic molecules, e.g. interfering RNA, to investigate the effects of candidate genes on cellular functions.		
F.4	Identification and monitoring of B or T cell populations as identified by flow cytometry and by their B cell or T cell receptor expression.	No	No
F.5	Measurement of gene expression in cells or tissues. Techniques frequently used include gene expression profiling on microarrays, quantitative RT-PCR, Western blotting, flow cytometry and ELISA assays.	No	No
F.6	Analysis of chromosomal abnormalities or mutations in malignant cells and non-malignant cells including FISH technology and DNA sequencing.	No	No
F.7	Assays of immune function of B-cells and T-cells, including intracellular cytokine staining, ELISPOT, quantitative RT-PCR for cytokines or other immune regulatory genes.	No	No
F.8	Analysis of antibody specificities in serum and antigen specificity of the B-cell receptor on cells. Techniques may include expression of antibodies in phage display systems, generation of antibodies in cell culture systems and use of such antibodies to screen for cognate antigens.	No	No
F.9	Transplantation of human cells into mice (xenograft model) to study disease biology and to investigate the effect of experimental therapy.	No	No

F.10	Measurements of drug concentrations, biologic molecules and disease markers in blood, serum, and plasma.	No	No

APPENDIX B: GVHD scoring scales

Acute GVHD Grading and Staging

Extent of Skin Involvement			
Stage	**Skin**	**Liver**	**Gut**
1	Rash on <25% of skin[a]	Bilirubin 2-3mg/dl[b]	Diarrhea >500mg/day[c] or persistent nausea[d]
2	Rash on 25-50% of skin	Bilirubin 3-6mg/dl	Diarrhea >1000mg/day
3	Rash on >50% of skin	Bilirubin 6-15mg/dl	Diarrhea >1500mg/day
4	Generalized erythroderma with bullous formation	Bilirubin >15mg/dl	Severe abdominal pain with or without ileus
Grade[e]			
I	Stage 1-2	None	None
II	Stage 3 or	Stage 1 or	Stage 1
III	X	Stage 2-3 or	Stages 2-4
IV[f]	Stage 4	Stage 4	X

a. Use "Rule of Nines"or burn chart to determine extent of rash.
b. Range given as total bilirubin. Downgrade one stage if an additional cause of elevated bilirubun has been documented.
c. Volume of diarrhea applies to adults. For pediatric patients, the volume of diarrhea should be based on body surface area. Downgrade one stage if an additional cause of diarrhea has been documented.
d. Persistent nausea with histologic evidence of GVHD in the stomach or deudenum.
e. Criteria for grading given as minimum degree of organ involvement required to confer that grade.
f. Grade IV may also include lesser organ involvement with an extreme decrease in performance status.

Reporting of Chronic GVHD (Sullivan KM, *Blood* 1981; 57:267.)

Limited: Localized skin involvement resembling localized scleroderma with or without liver involvement; no other organ involvement.

Extensive: Generalized skin and/or multiple organ involvement.

Report "limited" if chronic GVHD includes only localized skin involvement and/or liver dysfunction.

Report "extensive" if **any** of the following symptoms are attributed to chronic GVHD:

- Generalized skin involvement and/or liver dysfunction
- Liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis
- Involvement of the eye
- Involvement of the salivary glands or oral mucous membranes
- Involvement of any other target organ