CLINICAL RESEARCH PROJECT

Protocol #08-H-0046
IND 13,500
Product: Miltenyi CliniMACS® CD34 Reagent System + Unrelated Cord Blood Transplant
IND Sponsor-Investigator: Richard W. Childs, M.D.

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Title: Co-Infusion of umbilical cord blood and haploidentical CD34+ cells following nonmyeloablative conditioning as treatment for severe aplastic anemia and MDS associated with severe neutropenia refractory to immunosuppressive therapy

Other Identifying Words: Severe Aplastic Anemia (SAA), umbilical cord blood, haploidentical, nonmyeloablative

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Subjects of Study:	**Number**	**Sex**	**Age range**
Subjects:	Recipients: 40 Donors: Up to 60	either	Recipients: 4-55 inclusive Donors: 4-75 inclusive

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

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PRECIS

Severe aplastic anemia (SAA) and myelodysplastic syndrome (MDS) are life-threatening bone marrow disorders. For SAA patients, long term survival can be achieved with immunosuppressive treatment. However, of those patients treated with immunosuppressive therapy, one quarter to one third will not respond, and about 50% of responders will relapse.

Allogeneic bone marrow transplantation from either HLA-matched sibling or matched unrelated donor cures about 70% of patients with SAA and 30-60% of patients with MDS. Unfortunately, most patients with these disorders are not suitable candidates for hematopoietic stem cell transplantation (HSCT) due to advanced age or lack of a histocompatible donor. For such patients, transplantation using unrelated cord blood (UCB) has been shown to be a reasonable alternative transplant strategy. The advantage to UCB transplant is the ease and rapidity of availability, requirement of less than perfect HLA match, and lower rates of graft versus host disease compared to mismatched bone marrow or peripheral blood stem cell transplants. The major disadvantage of UCB transplantation in adults is the limited number of nucleated cells contained within the cord unit resulting in prolonged neutropenia and failure of engraftment which contributes to infection and transplant related mortality (TRM). In order to harness the advantage of UCB availability and to overcome the disadvantage of delayed neutrophil recovery, we propose to test whether co-administration of unrelated umbilical cord blood and a relatively low number of highly purified haploidentical peripheral blood CD34+ cells from a related donor might promote rapid engraftment and reduce TRM secondary to prolonged neutropenia associated with conventional UCBT.

This research protocol is therefore designed to evaluate the safety and effectiveness of co-infusion of unrelated umbilical cord blood and haploidentical CD34+ cells from a related donor following non-myeloablative conditioning for neutropenic patients with SAA or MDS with refactory anemia (RA) that has proven to be refractory to medical therapy. Subjects will receive a novel non-myeloablative immunosuppressive conditioning regimen of cyclophosphamide, fludarabine, horse ATG and one dose of total body irradiation (200cGy) followed by an infusion of the allografts. The haploidentical stem cell product will be T-cell depleted and enriched for CD34+ cells using the Miltenyi CliniMacs system. To reduce TRM secondary to prolonged neutropenia associated with conventional UCB transplantation, haploidentical CD34+ stem cells will be co-infused with a single UCB unit (serologically matched at ≥ 4/6 HLA loci).

The primary endpoint is donor engraftment by day 42 (defined as an ANC of >500 from either the haplo donor, the cord, or both combined). Secondary endpoints will include standard transplant outcome variables such as non-hematological toxicities, incidence and severity of acute and chronic GVHD, and relapse of disease. We will also evaluate ANC recovery (ANC > 500 cells/μl) at day 22, and 100 day and 200 day treatment related mortality (TRM) of this novel transplant approach. Health related quality of life will also be assessed pre-transplant, 30 and 100 days post-transplant, and every 6 months until 5 years post-transplant.

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SEE ALSO THE SUPPORTIVE CARE GUIDELINES NHLBI,
IRB reviewed 10/2003, 6/2006, and 1/2011

1.0 OBJECTIVES

1.1 Primary objective:

- To evaluate the potential of this modality to achieve donor engraftment in >80% of recipients by day 42 (defined as an ANC of >500 from either the haplo donor, the cord, or both combined).

1.2 Secondary objectives:

- To evaluate the day 22 ANC recovery rate (ANC $\geq$ 500 cells/μl)
- To evaluate the safety of this novel transplant regimen (non-hematologic toxicities)
- To evaluate day 100 and 200 TRM
- To determine the incidence and severity of acute and chronic graft-versus-host-disease (cGVHD) following this transplant regimen
- To evaluate the effect of a reduced conditioning transplant regimen on quality of life.

2.0 BACKGROUND

2.1 Aplastic Anemia (AA)

2.1.1 Pathophysiology

Aplastic anemia is a disease characterized by pancytopenia and a hypocellular bone marrow**.** Although the exact etiology of aplastic anemia is not known, clinical experiences and laboratory data suggest that the ultimate mechanism leading to development of bone marrow failure is immune-mediated. [1, 2] Suppression of hematopoiesis in aplastic anemia is likely secondary to specific populations of effector T-cells. Activated cytotoxic T-cells expressing HLA-DR, the IL-2 receptor, and IFN-γ are elevated in aplastic anemia patients and localized in the bone marrow.[3-5] Hematopoietic progenitor and stem cells are targets for the immune attack by these cells. The effects exerted by cytotoxic T-lymphocytes are at least in part due to Fas ligand-induced apoptosis of hematopoietic progenitor cells; IFN-γ, in addition to its intrinsic inhibitory activity on hematopoietic progenitor and stem cells, can induce over-expression of Fas on target cells.[6, 7] Despite its often acute presentation, aplastic anemia is now being recognized as a chronic disease with frequent flares of the immune process and the need for long-term immunosuppression. High resolution Vβ CDR3 analysis in patients with aplastic anemia shows significantly increased nonrandom skewing of the Vβ-chain families of the T cell receptor, suggestive of disease specific clonal expansion.[8]

2.1.2 Clinical consequences of aplastic anemia

Symptoms derive from low blood counts. Anemia leads to fatigue, weakness, lassitude, headaches, and in older patients dyspnea and chest pain. These manifestations are most commonly responsible for the clinical presentation. Thrombocytopenia produces mucosal bleeding. Petechiae of the skin and mucous membranes, epistaxis, and gum bleeding are frequent and early complaints. Bleeding can be brisk in the presence of accompanying physical lesions, as in gastritis and fungal infection of the lungs. The most feared complication of thrombocytopenia is intracranial hemorrhage. Bacterial and fungal infections in the setting of neutropenia are a major cause of morbidity and mortality, and are most often the causes of death in refractory cytopenias.

2.1.3 Treatment options for aplastic anemia

- ***Horse antithymocyte globulin (ATGAM®; h-ATG)*** is currently approved for the treatment of aplastic anemia by the Food and Drug Administration. The mechanism by which h-ATG improves bone marrow failure in aplastic anemia is not fully known. While it is generally believed that h-ATG administration leads to depletion of immune competent cells, its exact mechanism of action remains unclear. h-ATG preparations contain a variety of antibodies recognizing human T-cell epitopes, many directed against activated T-cells or activation antigens.[9, 10] After treatment with h-ATG, circulating levels of lymphocytes drop to 10% pretreatment level through a variety of mechanisms: Fc receptor complement-dependent lysis, opsonization and phagocytosis by macrophages; and immunomodulation leading to long-term depletion via antibody dependent cell-mediated cytotoxicity and activation-induced apoptosis. Although the decline in circulating levels of lymphocytes is transient, the number of activated T -cells is decreased for more prolonged periods of time; this effect is also reflected in decreased IFN-γ and possibly TNF production after h-ATG.[11, 12] Tissue culture preparations of peripheral blood lymphocytes treated with h-ATG produce hematopoietic colony stimulating factors, suggesting a possible stimulatory role in vivo. h-ATG binds to numerous other cell types in addition to lymphocytes, including cells of the bone marrow.[13-15] The response to h-ATG may be mediated by circulating factors produced in this immunologically activated state, although response to h-ATG has not correlated with severity or presence of clinical serum sickness.

- ***Rabbit ATG (Thymoglobulin®; r-ATG)*** is similar to h-ATG, except that gamma immune globulin is obtained by immunization of rabbits with human thymocytes. r-ATG contains cytotoxic antibodies directed against antigens expressed on T lymphocytes. r-ATG has been commercially available in Europe since 1985 and is currently licensed in more than 44 countries and has been used in more than 44,000 patients worldwide. In an Italian multi-center study, 30 aplastic anemia patients who failed h-ATG plus CsA received second line treatment with r-ATG and cyclosporine.[16]Overall response, defined as transfusion-independence was achieved in 23/30 (77%) patients after a median of 95 days, and overall survival was 93% with a median follow up of 914 days, with no patient having relapsed at time of publication. In our limited recent NIH experience, employing r-ATG as standard treatment in patients who had failed h-ATG, response rates have been less impressive: three partial responses; two late responses occurring more than six months after treatment and therefore of uncertain relationship to therapy; and nine patients without a response; one patient died of complications of pancytopenia soon after r-ATG treatment. Initial treatment with r-ATG has also been evaluated. Stein et al used r-ATG in combination with prednisone ± cyclosporine ± androgens in 57 patients with aplastic anemia, 30 being severe and 27 moderate.[17] Response occurred in 16 cases (28%), being more frequent in females, those with less than 6 months duration and with severe disease. Eight patients had previously received r-ATG and 2 (25%) responded.

- ***Cyclosporine (CsA)*** is a major immunosuppressive drug and probably secondary only to corticosteroids in world wide popularity in this role. In addition to its longstanding use in bone marrow and solid organ transplant recipients, CsA has been widely employed as an immunosuppressive drug in many autoimmune diseases. In contrast to ATG, CsA has a very selective inhibitory effect on T lymphocytes, suppressing early cellular response to antigenic and regulatory stimuli. By blocking expression of nuclear regulatory proteins, it leads to reduced T cell proliferation and activation with diminished release of cytokines such as interleukin-2 and interferon-γ. The addition of CsA to ATG has improved response rates to

70-80% and the 5 year survival in responding patients to 80-90%.[18, 19] CsA binds to intracellular receptors termed immunophilins, inhibiting in turn the activity of calcineurin, which results in the blocking of interleukin-2 production and T cell activation and proliferation. In vivo, CsA inhibits the release of IL-2 from activated T-cells and consequently decreases T cell proliferation.[20-22]

- ***Alternative immunosuppressive agents*** have been used with debated success. At Johns Hopkins, high dose **cyclophosphamide** without stem cell rescue produced hematologic responses in SAA patients similar to those seen with ATG combined with CsA, with no relapse or evolution to PNH or myelodysplasia being observed. [23] However, a prospective randomized trial conducted at the NHLBI, which compared ATG and cyclosporine to cyclophosphamide and cyclosporine, was terminated prematurely due to excessive toxicity, severe fungal infections and deaths in the group that received cyclophosphamide.[24] In contrast to the Hopkins experience, some of our patients relapsed or developed cytogenetic abnormalities. [25] The explanation for the increased toxicity seen in the cyclophosphamide treated patients is the serious immunosuppression and resulting neutropenia. The NIH is currently investigating the efficacy of **alemtuzumab (Campath®)** in subjects with MDS and SAA who have received no prior ATG based immunosuppressive therapy, in subjects who are refractory or had a suboptimal response to a course of horse ATG-based immunosuppression, and in subjects who have responded and relapsed following a prior course of ATG or are refractory to prior courses of horse and rabbit ATG.

2.1.4 Transplant options for patients with Aplastic anemia

- ***HLA- identical related donor transplantation for patients with SAA***: Allogeneic bone marrow transplantation from a histocompatible matched sibling is curative therapy in the majority of aplastic anemia patients who undergo the procedure.[26-29] Survival rates with allogeneic hematopoietic stem cell transplantation from a histocompatible sibling have been reported to be as high as 90% from a single institution and approximately 70% for registry data, which more likely reflects the general experience.[30, 31]

  High dose immunosuppression both eliminates active patient immunity that is involved in the pathogenesis of aplastic anemia and suppresses the recipient's immune system to prevent rejection of the graft. Cyclophosphamide as a single agent can be used to condition patients undergoing HSCT. The addition of ATG to cyclophosphamide conditioning appears to significantly reduce the risk of graft rejection. A report of 39 consecutive patients who received cyclophosphamide (total 200mg/kg in four daily doses) and ATG (total 90mg/kg in three doses using an HLA matched sibling donor) conditioning for a first transplant from an HLA matched sibling donor reported a graft rejection rate of only 5%. The 3 year survival rate was 92% compared to the 72% in matched historical controls receiving cyclophosphamide alone.[30] A more recent series of 81 patients with SAA undergoing this transplant approach reported 96% of the patients had sustained engraftment with 88% of patients being long term survivors at a median follow up of 9.2 years. [32]

  Patients with SAA who are older (i.e. >40 years of age), have failed prior immunosuppressive therapy, or who are heavily transfused have a higher risk of graft failure after HSCT. Minimizing the numbers of transfusions and using irradiated, leukocyte-depleted blood products appears to decrease this risk. Improved transfusion practices may be responsible, in part, for lower graft failure rates and better transplant outcomes observed in recent years.[27] Several strategies have been employed to decrease graft failure. One such approach is the addition of radiation, either total body, total lymphoid, or thoraco-abdominal.[33, 34]

However, radiation is associated with a higher risk of secondary malignancies [35-38], pneumonitis and chronic GVHD. The introduction of fludarabine in combination with cyclophosphamide with or without ATG allows for the elimination of irradiation from the conditioning regimen and was recently shown to result in excellent engraftment rates, even in heavily transfused and alloimmunized patients that have received prior immunosuppressive therapy [39-41]

- ***Matched unrelated donor transplantation for patients with SAA:*** Current practice guidelines do not recommend transplantation from an alternative donor as first-line treatment. However, only about one third of otherwise eligible transplant candidates will have an HLA-identical related donor. Alternative donor sources include HLA-mismatched related donors or a closely-HLA matched unrelated donors.[39] The probability of survival after unrelated donor transplantation is about half that observed in matched sibling transplants. Therefore, HSCT using unrelated donors is reserved for children identified to have a suitably matched unrelated donor that have failed a single course of immunosuppression or for adults who are refractory to multiple courses of ATG +/- alternative therapies such as androgens. Causes of transplant failure include a higher frequency of graft rejection, regimen-related toxicity (because of intensification of the conditioning regimens aimed at preventing rejection), and a higher incidence of acute graft-versus-host disease (GVHD) compared with HLA-identical sibling transplants. An analysis of 318 matched unrelated donor transplants followed over a median period of 5 years from 1988-1998 reported a graft failure rate of 15%, a 48% incidence of grade II -IV GVHD and a 5-year survival of 39%[42]. Many conditioning regimens for alternative donor transplants incorporate irradiation to facilitate engraftment. A prospective multicenter study conducted from 1994-2004 in 62 patients with aplastic anemia undergoing HLA matched unrelated donor HSCT determined that 200cGy (in combination with cyclophosphamide and ATG) was the minimal effective dose of total body irradiation required to achieve sustained engraftment without inducing prohibitive toxicity. Graft failure occurred in 2%, acute GVHD (grade II –IV) in 70%, chronic GVHD in 52%, and overall survival was 61%.[43]

  The EBMT-SAA Working Party recently reported results using a conditioning regimen substituting fludarabine for irradiation in 38 related and family mismatched donor transplants; graft rejection occurred in 18% with a 2-year survival rate of 73%.[44]

- ***Unrelated cord blood transplantation for patients with SAA***: Umbilical cord blood (UCB) is also increasingly being used as a source of stem cells, as this allows the transplantation of patients without an HLA-matched donor. A group in China treated nine young adults suffering from severe AA with umbilical transplants using cyclophosphamide and antilymphocyte globulin conditioning.[45] Seven of the nine patients had some level of engraftment, evidenced by the presence of stable mixed chimerism. After a median follow-up of 32 months, survival was nearly 80%. Successful complete chimerism was reported in a pediatric patient after an umbilical cord graft; stable engraftment was also achieved after UCB grafts in children with congenital AA in two other reports. Umbilical cord transplants therefore appear to be an option for patients with AA who lack a suitable HLA-matched donor, but more experience is needed to determine the long-term outcome of this approach. Transplantation using unrelated cord blood (UCB) has been shown to be a reasonable alternative transplant approach for patients with hematological disorders curable by transplantation who lack an HLA matched sibling to serve as a stem cell source. Advantage of UCB transplantation include the rapidity by which UCB units can be obtained, the requirement for less than a perfect HLA match, and lower rates of graft versus host disease associated with HLA mismatching compared to bone marrow or peripheral blood stem cell

transplants. The major disadvantage of UCB transplantation in adults is the limited number of nucleated cells contained within the cord unit resulting in prolonged neutropenia and failure of engraftment, which contributes, to infection and TRM.

The COBLT study was a prospective study of unrelated cord blood transplantation designed to better define the role of this stem cell source for subjects requiring unrelated allogeneic transplantation; 34 adult subjects (median age 34.5 years) were enrolled. Most of these patients had hematological malignancies and were transplanted with a single umbilical cord blood unit (containing greater than 10e7 nucleated cells per Kg of recipient weight) after a myeloablative conditioning regimen. For the primary end point, survival at 180 days was dismally low at 30%. Median time to neutrophil and platelet engraftment were unacceptably prolonged at 31 days (range, 13 to 55 days) and 117 days respectively and the primary graft failure incidence was 34%. Acute and chronic GVHD were the most common cause of death after CBT in this study.[46]

Although increasing the cell dose has been reported to improve engraftment kinetics, it is unclear whether higher cord blood cell doses will increase the percentage of subjects who will successfully engraft. Whereas doses of $< 1.5 \times 10^7$ total nucleated cells (TNCs) per kilogram are associated with a higher incidence of graft failures[47], Rubinstein et al. [48] did not detect a decrease in graft failures above a dose of 2.5×10^7 TNCs per kilogram, thus suggesting that there may be a threshold dose for successful engraftment. This study and previous CBT studies [46, 49-51]indicate that despite the slow engraftment times, approximately 85% to 90% of subjects undergoing CBT will eventually engraft.

It is now clear that the limited umbilical cord blood (UCB) cell dose is the major factor that compromises outcome in adult recipients of UCB transplantation. Therefore, to augment graft cell dose, combinations of 2 partially human leukocyte antigen (HLA) matched UCB units were evaluated prospectively in twenty-three patients with high-risk hematological malignancies.[52] Engraftment was quicker using this approach, with neutrophil recovery occurring at a median of 23 days (range, 15-41). Such “dual cord” transplants may shorten neutropenia compared to transplantation approaches using a single umbilical cord blood unit, but conditioning associated neutropenia of 22 days is still considerably longer than the median 12 days of neutropenia associated with the use of peripheral blood stem cell transplants.

There are currently no published prospective studies using dual cord transplants as a stem cell source in patients with severe aplastic anemia.

2.2 Myelodysplastic Syndrome (MDS)

2.2.1 Pathophysiology of MDS

The myelodysplastic syndromes are a heterogeneous group of clonal hematological disorders characterized by bone marrow failure, multiple refractory cytopenias (anemia, neutropenia, and thrombocytopenia) and morphologic evidence of marrow dysplasia (proliferation of myeloblastic leukemia cells). As a result, patients with MDS are at variable risk of progression to acute leukemia which is often refractory to standard treatment. These disorders may arise de novo or appear years after exposure to potentially mutagenic chemotherapy. The majority of patients with myelodysplasia have a hypercellular or normocellular bone marrow. In a minority of patients, less than 15%, a hypocellular bone marrow is found and the abnormality is referred to as hypoplastic myelodysplasia.[53] Hypoplastic MDS bears morphologic similarity to aplastic

anemia. In some patients with aplastic anemia who initially respond to immunosuppressive therapy, progression to MDS may occur also suggesting that these two entities may share a common pathophysiology, T-cell-mediated suppression of a primitive bone marrow stem cells.

2.2.2 Cytogenetic abnormalities present in MDS

Cytogenetic abnormalities are present in 40-70% of de novo MDS cases[54-58] . Many times, patients may present with cytopenias but minimal or no evidence of marrow dysplasia and a diagnosis of MDS is based on abnormal cytogenetics. Additionally, survival and the risk of leukemic transformation can be prognosticated based upon cytogenetic abnormalities. Deletion 5q, deletion 20q, or deletion Y, when occurring as the only cytogenetic abnormality are considered low risk MDS with the most favorable median survival (5.7 years) and lowest risk of progression to acute myeloid leukemia (AML). Complex karyotypes are associated with the poorest median survival (0.4 years) and greatest risk of progression to AML. Intermediate karyotypes such as trisomy 8 and other abnormalities that are not listed above fall in between with a median survival of 1.2 to 3.5 years.[58]

2008 WHO Classification Criteria for Myelodysplastic Syndromes

Disease	Blood Findings	Bone Marrow Findings
Refractory cytopenia with unilineage dysplasia (RCUD): Refractory anemia (RA) Refractory neutropenia (RN); Refractory Thrombocytopenia (RT)	Unicytopenia or Bicytopenia No or rare blasts < 1 x 10^9/L monocytes	Unilineage dysplasia only < 5% blasts < 15% ringed sideroblasts
Refractory anemia with ringed sideroblasts (RARS)	Anemia No blasts	Erythroid dysplasia only < 10% grans or megas dysplastic ≥15% ringed sideroblasts < 5% blasts
Refractory cytopenia with multilineage dysplasia (RCMD)	Cytopenias (bicytopenia or pancytopenia) No or rare blasts No Auer rods < 1 x 10^9/L monocytes	Dysplasia in ≥10% of cells in two or more myeloid cell lines < 5% blasts in marrow No Auer rods < 15% ringed sideroblasts
Myelodysplastic syndrome, unclassified (MDS-U)	Cytopenias No or rare blasts No Auer rods	Dysplasia in <10% of cells in one or more Lineages with an MDS-associated Cytogenetic abnormality < 5% blasts No Auer rods

2.2.3 Clinical consequences of MDS

Symptoms that derive from low blood counts include: anemia which leads to fatigue, weakness, lassitude, headaches, and in older patients, dyspnea and chest pain. These manifestations are most commonly responsible for the clinical presentation. Thrombocytopenia produces mucosal bleeding: petechiae of the skin and mucous membranes, epistaxis, and gum bleeding are frequent and early complaints. Bleeding can be brisk in the presence of accompanying physical lesions, as in gastritis and fungal infection of the lungs. The most feared complication of thrombocytopenia is intracranial hemorrhage. Bacterial and fungal infections in the setting of neutropenia are a major cause of morbidity and mortality and are often the cause of death in refractory cytopenia

associated with MDS. Patients with RA and RAS have a low risk of developing AML and their median survival is more than 30 months. The majority of these patients die of consequences of bone marrow failure or due to iron overload as a result of repeated blood transfusions. The median survival of patients presenting with RAEB and RAEB-t is generally shorter than 12 months.[59]

2.2.4 Treatment options for MDS

- ***Growth factors:*** Until recently, most patients were treated only with transfusions and growth factors to improve blood counts. While many people assume that the majority of patients die from leukemic transformation, progression to acute myeloid leukemia (AML) occurs in up to 40% of patients with MDS [60] but an equal proportion of patients die from bleeding or infection resulting from cytopenias. Additionally, symptoms of anemia, and frequent trips to the treating physician's office for blood and/or platelet transfusions significantly impact quality of life. In low risk MDS patients, erythropoietin provides a modest benefit for the treatment of anemia and many studies have shown a superior benefit when granulocyte stimulating factor (GCSF) is added to erythropoietin.[61-64] Additionally, GCSF alone can stimulate the production of neutrophils to prevent infectious complications from long-term neutropenia.[65, 66] Unfortunately, growth factors work only for a finite period of time and patients eventually succumb to anemia requiring transfusion support and prolonged neutropenia. The prognosis for patients with MDS/AML is very poor with high chemotherapy complication rates and a short duration of remission in responders.

- ***Immunosuppresive therapy:*** Indeed, antithymocyte globulin (ATG), the mainstay of non-transplantation therapy for aplastic anemia, has a role in certain patients with MDS. A report from the National Cancer Institute suggests that patients with MDS who are young and who have low platelet counts may benefit from a trial of ATG, with response rates as high as 50% .[67, 68] Interesting recent findings have added DR15 histocompatibility type as another predictor for response to ATG in MDS. [68, 69]Although it was originally thought that only patients with hypoplastic MDS would respond, it now seems clear that bone marrow hypoplasia is not a requirement to respond to rabbit or horse ATG, although patients with excess blasts rarely respond .[70]Investigators from Prague have shown that about 40% of patients with indolent-subtype MDS may respond to cyclosporine[71]. Although a large randomized trial testing the utility of ATG in MDS is ongoing, young patients with MDS who do not have excess marrow blasts can be considered for a trial of immunosuppression.

- ***Decitabine and Azacitidine (AzA-CR, 5-AZC):*** Azacitidine has been designated an orphan product for the use in the treatment of MDS but has shown only a 10-30% response rate.[72, 73]Its use requires close monitoring as cytopenias and central nervous system toxicities (lethargy, somnolence, confusion, coma) are frequent complications. The drug was recently licensed and is widely available to those who wish to use it off study. 5-azacytidine, can reduce transfusion requirements, delay time to leukemic transformation, and improve quality of life when compared to supportive care.[74] This trial will not exclude patients who have tried and failed Azacitidine, nor does it preclude its use in patients who have failed treatment.

  Decitabine, another hypomethylating agent, has shown to increase survival in patients with average and high risk MDS. A study of 170 average and high risk MDS patients showed an overall response of 17% with a 9% complete response and 13% hematologic response. In this population there was an increased leukemia free progression time. While overall survival is improved with both drugs, some patients are limited in their ability to tolerate these

medications secondary to worsening cytopenias. Additionally, all patients eventually progress or die of complications of their disease despite treatment.[74]

Revlimid® (Lenalidomide): Revlimid®, a drug structurally similar to Thalidomide, was approved by the United States Food and Drug Administration (FDA) on December 27, 2005 for the treatment of low-or-intermediate risk MDS associated with deletion of 5q, a chromosomal (cytogenetic) abnormality, with or without other cytogenetic abnormalities.[75]

2.2.5 Transplant options for patients with MDS

- ***Allogeneic stem cell transplantation for MDS.*** It is now well established that myelodysplastic syndromes (MDS) can be cured with allogeneic hematopoietic stem cell transplantation. Disease-free survival (DFS) ranges from 29% to 40%, with corresponding non-relapse mortality of 37 to 50% with the rate of relapse ranging from 23% to 48% with an HLA-identical sibling donor.[76-79] Longer disease duration before transplantation and older age is associated with an increased risk of treatment-related death after transplantation, particularly in those with life threatening cytopenias, thereby mandating consideration of transplant early in the course of the disease.[80] Outcome of SCT in patients with RAEB and RAEBt is less favorable than the outcome in patients with RA(RS), due largely to a higher risk of relapse. The European Group for Blood and Marrow Transplantation (EBMT) reported a 5-year actuarial relapse rate of 44% and 52% in 35 RAEB patients and 28 RAEBt patients.[81] The Fred Hutchinson Cancer Research Center (FHCRC) reported a 49% relapse rate for patients with excess of blasts compared to 4% for patients without marrow blast elevations, with actuarial DFS of 31% versus 54%, respectively.[82]

  This curative effect has been ascribed to the use of myeloablative chemo-radiotherapy and the antileukemic effect of the transplant (the graft-versus-leukemia [GVL] effect). The assumption that the intensive myeloablative preparative regimen is essential for the cure of the malignancy went unchallenged until the demonstration by Kolb et al [83], subsequently confirmed by numerous investigators, that donor lymphocytes alone exert a powerful antileukemic effect [84] in the context of patients relapsing with myeloid leukemias after BMT.[85-88] This observation has important implications:

  - First, it may be possible to cure some hematological malignancies with preparative regimens of lower intensity, designed to immunosuppress the recipient to allow lymphocyte and stem cell engraftment without major cytoreduction of the malignancy by myeloablative conditioning.[89]
  - Second, such low-intensity preparative regimens appear to have lower toxicity and may make transplantation appropriate in patients where procedural mortality is usually prohibitive, including patients with more indolent hematological diseases as well as patients with co-morbid diseases and older patients.[90]

Several groups, including our own, have begun to investigate this approach to improve the applicability and outcome following allogeneic BMT. Our experience at the NIH has included >150 subjects and has also been quite favorable, with a lower incidence of treatment-related complications than is typically observed with a standard "myeloablative" transplant approach. The application of reduced intensity conditioning (RIC) in MDS has had favorable results. Kröger, et al.[91] reported 37 patients with MDS, who were ineligible for transplants using conventional myeloablative conditioning. The reduced intensity conditioning consisted of fludarabine, a reduced dosage of busulphan and antithymocyte globulin. Overall TRM was 27%, with significantly higher mortality in those with poor-risk cytogenetics (75% versus 29%) or

with an HLA-matched unrelated donor (45% versus 12%). In total, 32% of patients relapsed, and actuarial disease free survival (DFS) at 3 years was 38% with a median follow-up of 20 months. A Spanish study showed a TRM of only 5% after transplantation of 37 patients with MDS and AML (median age: 57 years) utilizing a regimen of fludarabine and busulphan 10 mg/kg.[92] The 1-year progression-free survival was 66% with a corresponding frequency of disease-progression in patients with and without graft-versus-host disease (GVHD) of 13% (95% CI, 4%–34%) and 58% (95% CI, 36%–96%), respectively ($P = 0.008$). These results support the notion that a graft-versus-MDS/AML response is critical in reducing the risk of relapse after an RIC transplant. However the risk of relapse may be higher using RIC; a recent EBMT study reported a 54% relapse risk for 24 patients transplanted with RIC protocols, which translated into an increased hazard ratio (HR) of 6.0 ($p = 0.02$) for relapse compared to conventional transplantation regimens in a multivariate Cox model.[93]

In the current study, we will only include patients with refractory anemia (RA) orrefractory anemia with ringed sideroblasts (RARS) subtypes of MDS who have life-threatening neutropenia and who are at a lower risk of relapse with reduced intensity conditioning but are not candidates for a transplant utilizing an HLA matched related or unrelated donor.

- ***Stem cell transplantation using matched unrelated donors* for MDS.** Among 118 patients who received a stem cell transplant from an unrelated donor in the EBMT database, DFS at 2 years, relapse risk and TRM were 28%, 35% and 58%, respectively. The TRM was significantly influenced by age (younger than 18 years: 40%; 18–35 years: 61%; older than 35 years: 81%). Patients with more severe acute GVHD experienced a lower relapse risk, suggesting an increased graft-versus-MDS effect in these patients.[94]
- ***Stem cell transplantation using unrelated cord blood for MDS.*** At present, no case series reporting outcome of adult patients with MDS receiving unrelated cord blood transplants has yet been published. However, two recent retrospective analyses have been published comparing transplant outcome in adult patients with a variety of hematological malignancies undergoing umbilical cord blood (UCB) transplants versus matched unrelated donor (MUD) transplants. In an EBMT analysis of 682 patients undergoing UCB (n=98) vs MUD transplants, recipients of UCB transplants had a significantly lower incidence of acute GVHD (relative risk [RR] 0.57) with a similar incidence of CGVHD and similar leukemia free survival and overall survival.[50] An IBMTR analysis of 600 adult patients with leukemia undergoing transplantation from a matched unrelated donor (MUD) vs a single antigen mismatched unrelated donor versus a 1 or 2 antigen mismatched UCB transplant reported long-term leukemia free survival and overall survival to be slightly superior with MUD donors.[49] In this analysis, leukemia free survival and overall survival was similar among recipients of a single antigen mismatched unrelated donor transplant compared to UCB transplant recipients. These and other studies have now defined a clear role of UCB transplantation in the treatment of adults with hematological malignancies who lack an HLA matched sibling donor or fully matched unrelated donor. However, graft failure, which is most closely associated with patient size, age, and low cord stem cell doses, remains a significant problem that limits the applicability of this approach in adults. A number of approaches are being developed to overcome the obstacle of low-cell doses in adults, including the use of dual cord blood transplants in which UCB grafts are combined from different cord donors and the *ex vivo* expansion of umbilical cord progenitor cells. This protocol utilizes reduced intensity conditioning followed by cord blood transplantation with the co-infusion of haploidentical purified CD34+ cells to expedite neutrophil recovery. If successful, this approach could expand the application of allogeneic transplantation to a greater numbers of patients with MDS.

2.3 Haplo-identical Donor Transplantation

Another viable stem cell alternative for allogeneic transplantation is to utilize relatives who are only partially HLA-matched. These donors have one identical HLA haplotype and variable mismatches for zero, one, two or all three HLA-A, -B or DR loci of the unshared haplotype. Almost all patients have at least one HLA-haploidentical mismatched family member (parent, child, or sibling) who is immediately available as a donor. Unfortunately, in full haplotype mismatched transplants, a high frequency of alloreactive donor T cells in unmanipulated grafts exist that recognize mismatched MHC antigens, resulting in an extremely high incidence of severe, acute GVHD.[95-97] Although extensive T cell depletion prevents GVHD, the rejection rates rise steeply because the balance between competing host and donor T cells shifts in favor of the unopposed host-versus-graft reaction.[98, 99] Furthermore, T-cell depletion is associated with a greater risk of post-transplant viral infections and disease relapse.

2.4 Unrelated Cord Blood Combined with Haploidentical CD34+ Cell Infusion Transplantation

To harness the advantage of UCB availability and to overcome the disadvantage of delayed neutrophil recovery, a pilot study was conducted by Fernandez et al where 27 adult patients with high risk leukemia received single unit UCB transplantation co-administered with an infusion of a relatively low number of highly purified haploidentical peripheral blood CD34+ cells from a related donor.[100] Rapid engraftment of a purified CD34+ haploidentical cells was observed with the median time to the ANC >500/L of 10 days (range 9-36). This engraftment was much more rapid than is typically observed with a single UCB transplant (range 22-31 days). The time to a calculated cord blood ANC >500 cells/ul ranged from 13-55 days with a median of 22 days. With a median follow-up of 10 months (range 1-75 months), 19/27 patients survived. No major early infections, bacterial or fungal were observed and the need of antimicrobials during the period of neutropenia was lower than typically required for recipients of conventional UCB transplant. Chimerism studies revealed initial engraftment of the haplo-CD34+ cells, followed by engraftment of the cord unit resulting in dual chimerism. With time, chimerism from the haplo-CD34+ donor was observed to disappear with full chimerism from the UCB unit ultimately being observed. This approach appears to provide the benefit of rapid neutrophil engraftment that occurs as a consequence of the CD34+ cell infusion with the cord blood unit engrafting thereafter restoring defective cellular immunity that typically occurs as a consequence of the rigorous T-cell depletion associated with haploidentical stem cell infusion.

2.5 T Cell Depletion of G-CSF Mobilized PBSCs

T cell depletion is a technique to select and/or enrich specific cell populations from allografts. In this protocol we will use the Miltenyi CliniMacs immunomagenetic T cell depletion system that is widely used in Europe and available in the USA only under an IND/IDE. The CliniMACS® CD34 Reagent System is used *in vitro* to select CD34+ cells from heterogeneous hematological cell populations prior to transplantation. The Department of Transfusion Medicine (DTM) Cell Processing Section has evaluated Miltenyi CliniMacs CD34-positive selection as a method for T cell depletion of filgrastim (G-CSF) mobilized PBSC collections from normal donors. Results (mean ± SD) are as follows (n=11 products): CD34 % recovery = 60 ± 13 % (range 41-77%) and CD3+ T cell depletion = 5.3 ± 0.2 log (range 5.1-5.7 log) (personal communication with EJ Read). The T cell depletion is approximately one log greater than the Isolex CD34 positive selection only process, which routinely achieves up to 4 log T cell depletion.

2.6. Triple Drug Regimen for GVHD Prophylaxis

The prophylaxis of graft-versus-host disease (GVHD) has varied significantly among various studies of unrelated cord blood transplantation. Most have incorporated either tacrolimus or cyclosporine combined with other immunosuppressive agents such as mycophenolate mofetil, methotrexate, or a brief course of prednisone. At present, there is no accepted standard regimen for GVHD prophylaxis in this setting. This protocol will utilize tacrolimus in combination with mycophenolate mofetil and a brief course of prednisone as GVHD prophylaxis. [103]

3.0 SCIENTIFIC AND CLINICAL JUSTIFICATION

Severe aplastic anemia (SAA) is a life-threatening bone marrow failure disorder characterized by pancytopenia and a hypocellular bone marrow. Transplant and immunosuppressive treatment with ATG and CsA have dramatically changed the natural course of aplastic anemia, with 5 year survival of 75% in patients undergoing treatment[39]. However, of those patients treated with ATG/CsA, 25-33% will not respond; and about 50% of responders will relapse.[101] The introduction of more potent immunosuppressive medications such as alemtuzumab (Campath®) and r-ATG[16, 17] may have a favorable impact in response and relapse rates. However, definite evidence that these novel immunosuppressive therapies can reduce morbidity, induce response and alleviate relapse as compared to the conventional h-ATG/CsA treatment regimen currently does not exist. Patients who fail immunosuppression who have an ANC's <500 cells/µl are at high risk for death from opportunistic infections, particularly invasive fungal infections. Likewise, patients with refractory anemia (RA) or refractory anemia with ringed sideroblasts (RARS)subtypes of MDS who have severe neutropenia refractory to immunosuppression are also at high risk of death from opportunistic bacterial and fungal infections.

Allogeneic bone marrow transplantation from an HLA-matched sibling or matched unrelated donor offers the opportunity for cure in approximately 70-80% of patients. [30, 31] For patients lacking a matched related donor, a MUD transplant is the preferred therapy. However, many patients don't have a matched unrelated donor identified or don't have resources required for such a transplant.

Transplantation using unrelated cord blood (UCB) has been shown to be a reasonable alternative transplant approach for patients with hematological disorders curable by transplantation that lack an HLA matched sibling to serve as a stem cell source. The major disadvantage of UCB transplantation in adults is the limited number of nucleated cells contained within the cord unit resulting in prolonged neutropenia and failure of engraftment which contributes to infection and TRM. To harness the advantage of UCB availability and to overcome the disadvantage of delayed neutrophil recovery, a pilot study was conducted by Fernandez et al[100] in 27 patients (median age 29 years; range 16-60) with high risk leukemia who received a single unit UCB transplantation co-administered with an infusion of a relatively low number of highly purified haploidentical peripheral blood CD34+ cells from a related donor following a fully myeloablative conditioning regimen that included TBI. Rapid engraftment of purified CD34+ haploidentical cells was observed with the median time to the ANC >500 cells/ul of 10 days (range 9-36). This engraftment was much more rapid than is typically observed with a single UCB transplant (typically 22-31 days). Chimerism studies showed initial predominant chimerism in mononuclear cells and granulocytes being derived from the haplo-CD34+ cells followed by progressive replacement of these cells by cells derived from the cord blood unit. The time to a calculated cord blood ANC >500 cells/ul ranged from 13-55 days with a median of 22 days and a maximum cumulative incidence of the cord blood ANC being > 500 cells/µl of 0.93 (95% CI 0.83-1.00). Ultimately, full cord blood chimerism occurred in 25/27 patients at a median 55 days (range 11-96). Seventy-four percent of patients

developed acute GVHD although only 4 (14.8%) patients developed more severe grade III-IV GVHD. With a median follow-up of 10 months (range 1-75 months), 19/27 patients survived. Eight of 27 patients died as a consequence of non-relapse mortality. Causes of death included infection (n=3; 1 toxoplasmosis, 2 cytomegalovirus), vascular occlusive disease (VOD) (n=1), conditioning associated multisystem organ failure (MOF) (n=1), graft failure (n=1), and GVHD (n=2). No major early infections, bacterial or fungal were observed. The need of antimicrobials during the period of neutropenia was lower than typically required for recipient of conventional UCB transplantation.[100]

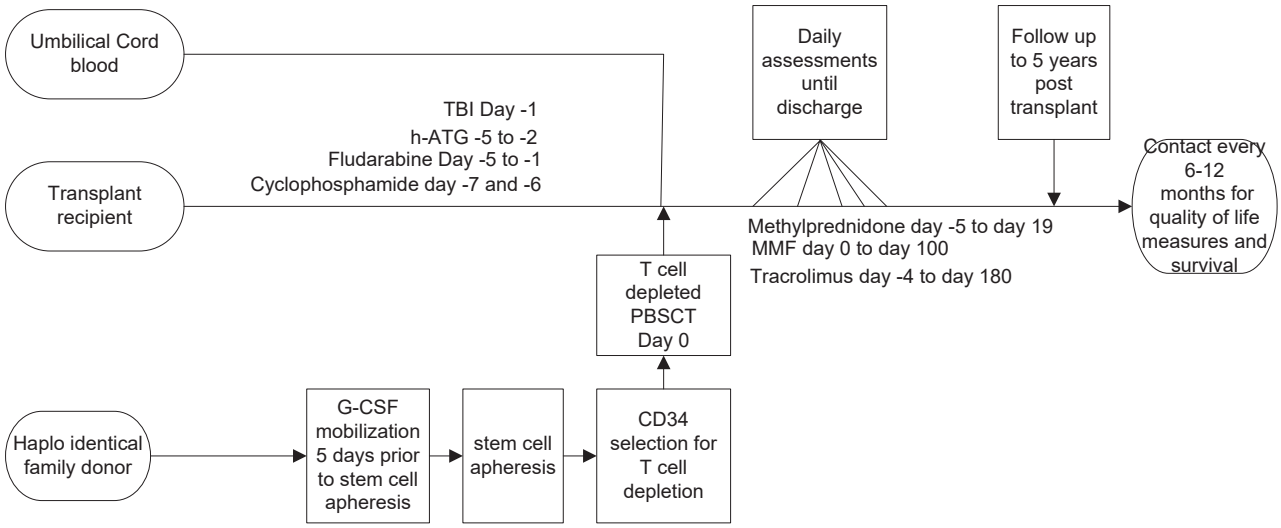
We therefore propose this research protocol of co-infusion of umbilical cord blood and haploidentical CD34+ cells following nonmyeloablative conditioning as treatment for subjects with SAA and RA and RARS subtypes of MDS refractory to immunosuppressive therapy. The study is being done to confirm the promising results published by Fernandez et al and to further establish whether this transplant approach can be effectively used in patients with aplastic anemia who are at high risk for graft rejection. Finally, it will investigate the engraftment potential of a novel nonmyeloablative conditioning regimen in this setting. Should engraftment rates be good (defined as an ANC > 500 in 80% of patients by day 42), this protocol will serve as a platform for subsequent trials investigating the impact of infusing in vitro expanded NK cells from the cord blood unit to reduce GVHD and to facilitate engraftment.

Justification for health related quality of life measures
The Food and Drug Administration (Johnson & Temple, 1985) and the Institute of Medicine (IOM, 2008) endorse Health Related Quality of life (HRQL) and its relevant dimensions as meaningful patient-reported indicators of the effects of treatment. The effects of allogeneic HSCT on HRQL dimensions are well established relative to standard conditioning regimens and disease populations. However, our understanding of the effects of reduced intensity conditioning regimens on specific disease populations such as aplastic anemia, bone marrow failure syndromes, PNH and myelodysplastic syndromes is less clear.

Therefore, we are proposing to incorporate a systematic evaluation of HRQL in adult individuals (English and Spanish when available) pre-transplant and throughout the trajectory of their recovery and follow-up post-transplant. Specifically, we request to incorporate patient-reported outcome measurements initially when the transplant recipient is on-study (pre-transplant prior to inpatient admission for transplant), then following transplant at day 30, day 100, every at each 6-month interval until the 5th year post transplant.

4.0 STUDY DESIGN

This is a phase II clinical trial designed to evaluate a novel non-myeloablative but highly immunosuppressive conditioning regimen consisting of cyclophosphamide, fludarabine, ATG and one dose total body irradiation, followed by an infusion of a stem cell product prepared from a haplo-identical donor using the Miltenyi CliniMacs system for CD34 selection, and a single ≥ 4/6 HLA antigen matched UCB unit in subjects with severe aplastic anemia or refractory anemia (RA) or refractory anemia with ringed sideroblasts (RARS)subtypes of MDS who have life-threatening neutropenia and who are not candidates for a transplant utilizing an HLA matched sibling or unrelated donor.



5.0 ELIGIBILITY ASSESSMENT

5.1 Inclusion Criteria-Recipient

5.1.1 Diagnosed with **severe aplastic anemia** characterized by all of the following:

a) Bone marrow cellularity <30% (excluding lymphocytes)

b) Transfusion dependence for platelets and/or RBCs

c) Neutropenia [(absolute neutrophil count < 500 cells/ μL) OR for patients receiving granulocyte transfusions, absolute neutrophil count < 500 cells/ μL before beginning granulocyte transfusions].

OR

Diagnosed with myelodysplastic syndrome characterized by refractory anemia OR refractory anemia with ringed sideroblasts (RARS) and at least one of the following:

a) Neutropenia [(absolute neutrophil count < 500 cells/ μL) OR for patients receiving granulocyte transfusions, absolute neutrophil count < 500 cells/ μL before beginning granulocyte transfusions] and history of 1 or more opportunistic infections related to neutropenia. Or

b) History of severe aplastic anemia transformed to MDS

5.1.2 Intolerance of or failure to respond to standard immunosuppressive therapy.

5.1.3 Availability of at least one HLA- haploidentical (i.e. ≥ 5/10 and ≤ 8/10 HLA match) related donor (HLA-A, B, C, DR, and DQ loci) who is available to donate CD34+ cells (4-75 years old).

5.1.4 Availability of at least one 4/6 HLA-matched (HLA-A, B, and DR loci) cord blood unit from the National Marrow Donor Program (NMDP). The cord blood unit must contain a minimum TNC (prior to thawing) of at least 1.5 x 10^7 cells per kilogram of recipient body weight with the following exception: if the minimum criterion of TNC is not met the cord unit must contain at least 1.7 x 10^5 CD34+ cells/kg (prior to thawing).

5.1.5 Ages 4-55 years inclusive.

5.1.6 Ability to comprehend the investigational nature of the study and provide informed consent. The procedure will be explained to subjects aged 4-17 years with formal consent being obtained from parents or legal guardian.

5.1.7 Telomere Length Testing.

5.1.8 In patients where a suspicion for a familial bone marrow failure syndrome (BMFS) exist, TERC and TERT mutation testing will be performed on protocol 04-H-0012 or performed elsewhere prior to enrolling on 04-H-0012.

5.2 Exclusion Criteria – Recipient (any of the following)

5.2.1 Availability of an HLA identical or 9/10 HLA matched (HLA A, B, C, DR, and DQ loci) - relative to serve as a stem cell donor.
5.2.2 The patient is deemed to be a candidate for a 6/6 HLA matched unrelated stem cell transplant (availability of a donor and resources required for such a transplant).
5.2.3 ECOG performance status of 2 or more
5.2.4 Major anticipated illness or organ failure incompatible with survival from transplant.
5.2.5 Severe psychiatric illness. Mental deficiency sufficiently severe as to make compliance with the transplant treatment unlikely and making informed consent impossible.
5.2.6 Positive pregnancy test for women of childbearing age.
5.2.7 HIV positive.
5.2.8 Diagnosis of Fanconi's anemia (by chromosome breakage study)
5.2.9 Diffusion capacity of carbon monoxide (DLCO) <40% using DLCO corrected for Hgb or lung volumes (patients under the age of 10 may be excluded from this criterion if they have difficulty performing the test correctly and thus are unable to have their DLCO assessed) using DL Adj and DL/VA/Adj.
5.2.10 Left ventricular ejection fraction < 40% (evaluated by ECHO)
5.2.11 Transaminases > 5x upper limit of normal (when transaminases are elevated, the subject may be excluded at the discretion of the PI).
5.2.12 Serum bilirubin >4 mg/dl.
5.2.13 Creatinine clearance < 50 cc/min by 24 hr urine collection (adjusted for body surface area, i.e.50 ml/min/1.73m^2).
5.2.14 Serum creatinine > 2.5 mg/dl
5.2.15 Failure to collect an adequate number of CD34+ cells (i.e. $\geq 2 \times 10^6$ CD34+ cells/kg) for transplantation from the subject's haploidentical relative.
5.2.16 Presence of an active infection not adequately responding to appropriate therapy.
5.2.17 History of a malignant disease liable to relapse or progress within 5 years.

5.3 Inclusion Criteria- Related Haploidentical Donor Donating Purified CD34+ Cells

5.3.1 HLA mismatched family donor ($\geq$ 5/10 and $\leq$ 8/10 HLA match (HLA-A, B, C, DR, and DQ loci)) who is available to donate CD34+ cells.
5.3.2 Ages 4-75 inclusive
5.3.3 Weight $\geq$ 15 kg.
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 who is not the recipient of the transplant and informed assent. The process will be explained to the minor on a level of complexity appropriate for their age and ability to comprehend.
5.3.5 If there is a suspicion of familial BMFS in the recipient, then the donor must have undergone genetic testing for genes associated with BMFS - performed at a CLIA-certified laboratory, prior to enrolling in this protocol.

5.4 Exclusion Criteria – Related Donor (any of the following)

5.4.1 Pregnant or breastfeeding.
5.4.2 A suitable adult haplo identical donor is available.
5.4.3 Unfit to receive filgrastim (G-CSF) and undergo apheresis (history of stroke, MI, unstable angina, uncontrolled hypertension, severe heart disease or palpable spleen).
5.4.4 HIV positive (Donors who are positive for HBV, HCV or HTLV-I/II, T.cruzi [Chagas] may be used at the discretion of the investigator following counseling and approval from the recipient).
5.4.5 Sickling hemoglobinopathies including HbSS or HbSC. Donors with HbAS are acceptable.
5.4.6 Severe psychiatric illness. Mental deficiency sufficiently severe as to make compliance with the BMT treatment unlikely, and making informed consent impossible.
5.4.7 Screening test positive for Chagas disease (Trypanosoma cruzi /T. cruzi/trypanosomiasis) confirmed by the Center for Disease Control (CDC).

6.0 CLINICAL EVALUATION OF THE RECIPIENT

Bone marrow aspirates will be read by a hematologist. Samples will be ordered and tracked through the CRIS 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.

6.1 Pre-Study Evaluation and Procedures

Lab test results and consults will be performed within 28 days of enrollment (unless otherwise specified); bone marrow evaluations will be performed within 3 months of enrollment; and pregnancy test will be performed within 72 hours of conditioning.

6.1.1 History and physical exam including ECOG assessment
6.1.2 Temperature, pulse, blood pressure, respiratory rate, height and weight
6.1.3 CBC with differential
6.1.4 Reticulocyte count
6.1.5 Acute care, mineral panel, hepatic panel, uric acid CK
6.1.6 Coagulation (PT, PTT)
6.1.7 Lipid panel
6.1.8 Prealbumin
6.1.9 C-reactive protein
6.1.10 Iron panel (ferritin, transferrin % saturation)
6.1.11 Folate level, B12 level
6.1.12 Thyroid function (TSH, T4), Adrenal function (FSH, LH)
6.1.13 Gonadal function (testosterone, estradiol), cortisol
6.1.14 Growth hormone
6.1.15 IgG
6.1.16 Lymphocyte Phenotyping TBNK
6.1.17 Isohemagglutinin titer
6.1.18 Hemoglobin electrophoresis
6.1.19 PNH flow cytometry of the peripheral blood for GPI anchored proteins on neutrophils
6.1.20 growth chart, (<18 years of age)
6.1.21 bone age (prepubertal children; within 3 months)
6.1.22 Infectious disease screens:
Anti-HBs antibody
HBsAg

Anti-HBc antibody
Anti-HCV Antibody
Anti –HTLV1/2 antibody
Anti HIV ½ antibody
West Nile Virus
HIV-1/HCV/HBV NAT
T.cruzi Antibody
Anti- HSV Type ½ Antibody,serum
Anti CMV IgG,IgM
Anti EBV antibody panel
Anti- Toxoplasma IgG
Anti-ToxoplasmaIgM antibody (Mayo)
Anti-Adenovirus Antibody
HHV6 PCR Quantitative, Blood
RPR
Anti Varicella Zoster Virus Antibody IgG, IgM
PPD

6.1.23 High Resolution HLA-A, B, C, DR, DQ typing (once)

6.1.24 Antibody screen for HLA alloantibodies (once)
HLA Antibody screen-Class 1A, B, C
HLA Antibody screen-Class 2 DRB1, DQB1
KIR Genotype (call 301-496 8852) for approval prior to ordering
Sequenced based HLA-C, ***this procedure requires advance approval by HLA supervisor prior to ordering (301-496-8852)***

6.1.25 Red cell ABO, Rh antibody screen, DAT (direct antiglobulin test)

6.1.26 Type and antibody screen

6.1.27 Direct Antiglobulin Screen

6.1.28 Short tandem repeats (STR) profile (once)

6.1.29 Chromosomal breakage studies to rule out Fanconi anemia (once)

6.1.30 Telomere length testing

6.1.31 FISH for MDS (if clinically indicated)

6.1.32 Quantitative immunoglobulins (IGG, IGA, IGM)

6.1.33 Dexa scan (within one year) radius, femur, spine

6.1.34 24-hour urine collection for creatinine clearance.
Urinalysis (includes microscopic)

6.1.35 For females of childbearing potential: pregnancy test (serum or urine)
Human Chorionic Gonadotropin, Preg
Pregnancy Test, Urine

6.1.36 Bone marrow aspirate and biopsy with cytogenetic analysis (morphology, cellularity, percentage of blast cells, and/or chromosomal analysis by PCR) as appropriate to stage and classify underlying disease

6.1.37 Chest radiograph (if patient does not have a chest CT)

6.1.38 Pulmonary function tests (patients under the age of 10 in which it has been established that they have difficulty performing the tests correctly will not be required to have their pulmonary function assessed.)

6.1.39 Sinus CT scan and other body CT scans as medically required

6.1.40 Cardiac function: EKG, ECHO
In addition, all subjects age 50 or greater or subjects age 40 or over with one of the following risk factors: a history of high blood pressure or increased cholesterol, family history of coronary disease, smoking or diabetes, will have a baseline cardiac workup, which will include:

1. Stress nuclear perfusion imagery (sestamibi) (if clinically indicated)
2. Cardiac consultation (all subjects age 50 and over, within 6 months of conditioning)

6.1.41 Nutritional assessment, as needed (within 6 months of conditioning)
6.1.42 Dental consult, as needed (within 6 months)
6.1.43 Social worker interview
6.1.44 Ophthalmology consultation (within 6 months of conditioning)
6.1.45 Discussion of the potential for infertility (within 6 months of conditioning)
6.1.46 Durable power of attorney form completed (within 6 months of conditioning)
6.1.47 Testing of genes related to inherited BMFS performed at a CLIA-certified laboratory - hTERC and hTERT, GATA2 mutations to be conducted in patients where a suspicion for familial bone marrow failure syndrome exist, on protocol 04-H-0012 or elsewhere prior to enrolling on 04-H-0012 to be eligible for this protocol (once)
6.1.48 HCT Comorbidity Index

6.2 In-Patient Monitoring (day -7 to discharge [approximately day 30])

* As the subject's condition stabilizes and they approach hospital discharge this monitoring frequency may decrease as clinically indicated (testing marked with an asterisk)

- Interim clinical assessments (daily while in patient)
- Temperature, pulse, blood pressure, respiratory rate, weight (*daily)
- CBC with differential (*daily)
- Acute care, mineral panel, hepatic panel, uric acid CK (*daily)
- Reticulocytes (*twice weekly)
- C-reactive protein (*weekly) if clinically indicated
- Pre-albumin (*twice weekly) if clinically indicated
- Lipid panel (*twice weekly) if clinically indicated
- Coagulation (PT, PTT) screen (*twice weekly) if clinically indicated
- Type and screen (*q4 days) only if indicated (patient still requiring transfusions)
- CMV, EBV (twice weekly)
- Toxoplasma gondii PCR, BLD (weekly if clinically indicated)
- HHV6, adenovirus blood PCR and urine PCR surveillance (weekly)
- BK blood PCR and urine PCR (*) (weekly if clinically indicated)
- Repeat pulmonary function tests (if patient is stable enough to perform PFTs): (Day 30 +/- 7 days) (patients under the age of 10 in which it has been established that they have difficulty performing the tests correctly will not be required to have their pulmonary function assessed.)
- PNH flow cytometry (every 2 weeks until negative for a PNH clone x2)
- Bone marrow aspirate or peripheral blood to quantitate engraftment (chimerism) in the lymphoid and myeloid cell lines day 15 +/- 7 days and day 30 +/- 7 days or when clinical conditions warrant testing.
- Drug levels where appropriate (e.g., gentamicin, vancomycin, tacrolimus (daily while inpatient, mycophenolate)
- TBNK Lymphocyte Phenotyping (Day 30 +/- 7 days)
- IgG (Day 30 +/- 7 days)
- Pregnancy test for females of childbearing potential (serum or urine) (within 72h of conditioning)
  - Human Chorionic Gonadotropin, Preg

- Pregnancy Test, Urine

6.3 Follow Up Period from Hospital Discharge to Return Home (about days 30- 180)

At least weekly (+/- 5 days) unless otherwise noted:
NOTE: If patients have had extended stays past day 180, visits may decrease, some tests listed below may not be medically indicated at the frequency listed. If this is the case, notes will be made in the charts.

- Clinical assessment
- Temperature, pulse, blood pressure, respiratory rate, weight
- CBC with differential
- Acute care, mineral panel, hepatic panel, uric acid CPK
- Reticulocyte count
- C-reactive protein (if clinically indicated)
- Ionized magnesium (if clinically indicated)
- Coagulation screen (if clinically indicated)
- EBV, CMV, PCR quantitative blood (twice weekly)
- HHV6 and adenovirus surveillance (weekly)
- BK blood PCR and urine PCR, andnovirus urine (if clinically indicated)
- DAT (direct antiglobulin test) and type and screen (as clinically indicated)
- Bone marrow aspirate or peripheral blood to quantitate engraftment (chimerism) in the lymphoid and myeloid cell lines day 42 (+/- 3 days), 60 (+/- 3 days) and 100 (+/- 7 days) or when clinical conditions warrant testing (for example, patients with MDS done at 30 +/-7 days, 100 +/- 14 days and 6 months +/- 30 days)
- Pregnancy test (at least monthly +/- 1 week) (for females of childbearing age) (as clinically indicated)
- Drug levels as appropriate (i.e.tacrolimus, MMF)
- TBNK Lymphocyte Phenotyping (Day 30, 60, and 100 +/- 7days)
- Vitamin D (Day 100 +/- 7 days)
- Iron Panel (Day 100 +/- 7 days)
- IgG (Day 30, 60, and 100 +/- 7 days)

6.4 Follow Up After Return Home

Subjects and/or their referring physician will remain in contact with the NIH and are asked to return to the NIH or send the results of clinical assessments and/or laboratory testing at least every 3 months (+/- 2 weeks) for 2 years, then every 6 months (+/- 1 month (national) and +/- 3 months for international patients) to 5 years. Additional labs/tests may be done as clinically indicated.

- Clinical assessment at NIH only
- CBC with differential
- Acute care, mineral panel, hepatic panel, uric acid CPK
- CD4/CD8 on day 180 (± 30 days) and day 360 (± 30 days)
- TBNK Lymphocyte Phenotyping (For all NIH visits)
- Vitamin D Day 180 (± 30 days)
- Iron Panel (NIH visits only)
- IgG (NIH visits only)
- Lipid panel (NIH visits only)

- Reticulocytes (NIH visits only)
- Chest radiograph (when clinically indicated)
- Pulmonary function tests (every 3 months x 2 years then every 6 months until 5 years after transplant then annually) (patients under the age of 10 in which it has been established that they have difficulty performing the tests correctly will not be required to have their pulmonary function assessed.) (± 30 days)
  NOTE: International patients who do not have the means to travel every 3 months after discharge home nor the money to obtain a PFT at home will not be required to have their pulmonary function assessed. Also, international patients who are stable enough to only need to return every 12 months may have the PFTs at PI's discretion and the PI will document when the patient will return.
- EBV, CMV, HHV6 and adenovirus surveillance by PCR (as clinically indicated)
- TBNK Lymphocyte Phenotyping (NIH visits only)
- IgG (NIH visits only)
- Bone marrow biopsy and aspiration with cytogenetics (for MDS patients only: 6 months, 1 year and then yearly until 5 years, for AA at 1 year follow up and then at the discretion of the PI). For bone marrows +/- one month and +/- 3months for international patients
- PNH flow cytometry of the peripheral blood for GPI cells (until normal x2)
- Ophthalmologic exam (yearly)
- Cortisol, TSH, T4, FSH, LH, and testosterone/estradiol (yearly)
- Growth hormone, growth chart, bone age (prepubertal children) (yearly)
- Health related quality of life measures (adults only, +/- 1 month): PROMIS 29, PROMIS v 1.0 Applied Cognition-General Concerns-Short Form, FACT-BMT, and Sexual Functioning Questionnaire (SFQ) screening questions 1-3 and subscales (Medical Impact, Satisfaction and Problems), Chronic Graft vs. Host Disease measure (every year until 5 years post-transplant).
- DEXA scan (radius, femur, spine AP) (yearly)

After 5 years, annual follow-up with the recipient and/or the referring physician will continue indefinitely to provide post-transplant standard of care and collect outcome and survival data as required by the National Marrow Donor Registry.

7.0 TREATMENT PLAN

7.1 Apheresis of Transplant Recipient for Lymphocytes (optional)

One cell collection of 10^{10} cells by apheresis for cryopreservation for studies of donor T cell alloresponses and post-transplant studies will be requested prior to transplantation.

7.2 Central Venous Line Placement

Central Venous Catheter Placement, when indicated, will be performed by the NIH Procedures, Vascular Access and Conscious Sedation (PVCS) Service. A separate consent will be obtained at the time of the procedure.

7.3 Infection Prophylaxis and Treatment

- ***Pneumocystis pneumonia* and *toxoplasmosis prophylaxis*:** PCP and toxoplasmosis prophylaxis will be given according to the NIH Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant Recipients.

- ***Strongyloides prophylaxis*:** Prophylaxis will be given according to the NIH Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant Recipients.

- ***Antiviral prophylaxis:*** Acyclovir or its equivalent will be given according to adult and pediatric doses found in the NIH Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant Recipients and administered for at least 1 year in recipients regardless of HSV serology status.

- ***Antibacterial prophylaxis*:** Bacterial prophylaxis to cover pseudomonas species (i.e. IV ceftazidime, meropenem, etc) will be given following initiation of the conditioning regimen in all recipients with an ANC< 200. Prophylactic antibiotic therapy will continue until the ANC recovers to >500.

- ***EBV, CMV, HHV6 and adenovirus monitoring and treatment:*** Subjects will be monitored for EBV, CMV, HHV6 and adenovirus PCR in the blood at baseline, then weekly until day 180 (±30 days). Thereafter, monitoring will be performed as clinically indicated.
    - EBV reactivation will be treated with rituximab
    - CMV reactivation will be treated according to the NIH supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant Recipients.
    - Adenovirus infection will be treated with cidofivir when clinically indicated. HHV6 reactivation will not be treated unless there exists compelling evidence for clinical disease related to HHV6; such recipients may receive foscarnet upon the advice of NIH Infectious Disease consultants.

7.4 Fever Regimen (See NIH Supportive Care Guidelines for Allogeneic Hematopoietic Stem Cell Transplant Recipients**)**

7.5 Neutropenia Regimen

- Filgrastim (G-CSF) will be initiated day +1 at a dose 5 mcg/kg day sq until neutrophils recover to ≥ 500 cells/ul. G-CSF administration will continue for 1 to 2 days after the ANC is >500 cells/μL based on physician's discretion and clinical judgment. Doses may be rounded +/- 10% to available vial sizes. G-CSF will be given subcutaneously as the preferred route and IV as the alternative route based on physician discretion.

7.6 Preparative Regimen

The preparative regimen will not be initiated until the cord blood unit obtained from the NMDP to be used for transplantation has been transferred to the NIH Department of Transfusion Medicine.

- Cyclophosphamide 60 mg/kg/d IV day -7, -6
- Fludarabine 25 mg/m^2/d IV day -5, -4, -3, -2, -1
- Antithymocyte globulin 40mg/kg IV days -5, -4,-3,-2 for a total dose of 160 mg/kg.

Subjects will receive pre-medication prior to infusion as follows:

- IV or oral diphenhydramine 25 to 50 mg (children 4-12 yrs 12.5-25 mg) and
- oral acetaminophen 650 mg (children 4-12 yrs: 325 mg)

Oral prednisone at 1 mg/kg/day (or IV methylprednisolone if clinically indicated) will begin on day -5 prior to the first dose of h-ATG for serum sickness prophylaxis, and will be continued at this dose for 10 days; thereafter, prednisone will be slowly tapered over the next 2 weeks until it is discontinued on day +19. Those recipients who develop serum sickness may require a longer tapering schedule and will be dosed individually as clinically indicated.

Infusions reactions will be treated symptomatically (e.g., anti-emetics, IV fluid hydration, acetaminophen, antihistamines, inhaled bronchodilators, meperidine).

In case of moderate or severe reactions hydrocortisone will be given and the infusion will be discontinued and restarted at a slower rate once the symptoms have subsided. The patient is often treated in the ICU and may require pressors. The patient may require have one or two days of rest prior to restarting the ATG necessitating that the conditioning regime and radiation may be delayed by 1 – 3 days. If this happens, the day of the transplant will be delayed by the number of days that the conditioning was delayed (by up to 3 days). If a recipient has a persistent severe infusion reaction that does not respond to measures to ameliorate the signs/symptoms associated to the infusion, the infusion will be discontinued and further study participation will continue as planned with the exception that no further ATG will be administered.

- **Total body irradiation (TBI)**: Day -1: All recipients should be treated with a linear accelerator using energies higher than 4MV. Recipients will be treated with TBI to a total dose of 200 cGy delivered in a single fraction over 30 minutes on Day -1 in the Department of Radiation Oncology. Equally weighted opposed lateral beams will be used to encompass the total body with the patient positioned supine. TBI will be delivered with lateral fields using extended SAD values of 600cm. Tissue compensators will be used if clinically appropriate. Gonadal shielding will not be used. 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.

7.7 Stem Cell Transplantation Day 0

7.7.1 Cord blood unit:

Minimum total nucleated cells (TNC) (prior to thawing) of 1.5 x 10^7 per kilogram of recipient body weight with the following exception: if the minimum criterion of TNC is not met the cord unit must contain at least 1.7 x 10^5 CD34+ cells/kg (prior to thawing). For cord blood products, there is no upper limit (maximum TNC) for the UCB unit since cord units are inherently limited in their number of TNCs and studies to date have not identified any adverse related outcomes associated with using higher cord blood cell doses.

Confirmatory typing reveals a minimum antigen level match in 4/6 HLA loci. Shipment of the cord blood unit will not be requested until the DTM confirms that the G-CSF mobilized T-cell depleted graft collected from the haploidentical donor contains a suitable number of CD34+ cells ($\geq$2 x 10^6/kg) and CD3+ T-cells (<1 x 10^4 CD3+ cells/ kg) (i.e. technical failure of the depletion process has not occurred).

7.7.2 Haploidentical donor:

The target, minimum and maximum cell doses (given in terms of recipient body weight) are:

CD34+ cells	Target dose: 3 x 10^6/kg
Minimum for transplant	2 x 10^6/kg
Maximum	5 x 10^6/kg
CD3+ cells	Target dose: 5 x 10^3/kg
Maximum	1 x 10^4/kg
Minimum	None

If the final CD3+ dose is greater than 1 x 10^4/kg the CD34+ dose will be reduced to achieve the targeted CD3+ dose of 5 x 10^3/kg

If the final CD3+ dose is greater than 1 x 10^4/kg after reducing the CD34+ dose to < 2 x 10^6/kg, this will be designated a technical failure of the depletion process. In this event, the subject will not be enrolled. The recipient will then be counseled regarding further available treatment options at the NIH or referred back to his/her primary care physician, whichever is in the best interest of the subject.

7.8 GVHD Prophylaxis:

Tacrolimus: Tacrolimus will be initiated at a dose of 0.03 mg/kg/day by continuous intravenous infusion over 24 hours *s*tarting on day -4. The tacrolimus dose will be adjusted to obtain goal tacrolimus therapeutic drug levels of 11-15 ng/mL. When the recipient is able to take oral medications, the 24-hour dose of tacrolimus will be converted to oral dosing (generally using a 1:3 or 1:4 IV to PO conversion or upon consultation with the clinical pharmacist) and administered in two divided doses every 12 hours. Tacrolimus will be continued for at least 6 months. NOTE: there may be some circumstances when it is medically indicated to stop tacrolimus before the time frame indicated here.

Mycophenolate mofetil (MMF): 1 gram (or approximately 15mg/kg/dose) administered orally or intravenously twice daily starting day 0 and continued for 100 days (possibly longer depending on PI's discretion). NOTE: there may be some circumstances when it is medically indicated to stop MMF before the time frame indicated here.

Prednisone: Oral prednisone at 1 mg/kg/day will begin on day -5 prior to the first dose of h-ATG for serum sickness prophylaxis, and will be continued at this dose for 10 days; thereafter, prednisone will be slowly tapered over the next 2 weeks until it is discontinued on day +19. Prednisone may be converted to an equivalent dose of methylprednisolone for intravenous administration if the recipient is unable to take oral medication.

7.8 Transfusion Support: Filtered and irradiated blood products as needed.

7.9 Nutrition: Parenteral nutrition will be instituted as necessary.

7.10 Hospital Discharge: The recipient will be in the hospital for about 4 weeks and will be discharged when clinically indicated to follow up as an outpatient (resolution of neutropenia in

the absence of fever and uncontrolled infection or other life threatening conditions that would require continued hospitalization).

7.11 Discharge Home: The recipient will remain in the vicinity of the NIH through the day 180 (±30 days) post-transplant assessments. Thereafter, recipients in stable condition without uncontrolled infection, GVHD, and/or refractory cytopenias will be eligible to be discharged home. Recipients may require a longer stay in the NIH vicinity if clinically indicated.

7.12 Health Related Quality of Life Measures (adults only):

PROMIS 29-Profile v1.0 and PROMIS v1.0-Applied Cognition-General Concerns-Short Form

Patient-Reported Outcomes Measurement Information System (PROMIS ®), is an initiative based on an NIH grant to establish and provide the public a free, reliable and validated source for commonly used measures of patient-reported outcomes (PROMIS, 2011). The PROMIS item databanks have been tested for reliability and comparability to more established measures of these same content areas by NIH scientists and select US academic institutions using Item-Response Theory (Baker, 2004).

The PROMIS 29 item profile instrument is a collection of profile short forms assessing seven primary HRQL domains (depression, anxiety, physical function, pain interference, fatigue, sleep disturbance, and satisfaction with participation in social roles). The PROMIS Applied Cognition-General Concern measure assesses problems with memory and concentration (Riley et al., 2010).

Functional Assessment of Chronic Illness Therapy-BMT (FACT-BMT)

The Functional Assessment of Cancer Therapy –BMT version 4 (FACT-BMT) measures disease specific QOL in transplant recipients. The FACT-BMT is an extended version of The Functional Assessment of Cancer Therapy –General (FACT-G) with 12 additional items that are transplant specific (Cella, 2004). The FACT-BMT has demonstrated validity and has evidence of internal consistency and reliability in the BMT population and in Spanish speaking oncology patients (Cella, 2004; Cella et al., 1998).

Sexual Functioning Questionnaire (SFQ) [subscales: Medical Impact and Problems, gender specific version]

The Sexual Functioning Questionnaire is a 30-item measure, self-report questionnaire (Syrjala, 2000). For this study we will use screening questions 1-3, the 9-item Problems, 2-item Satisfaction, and the 5-item Medical Impact subscales. Respondents will be given an option to "opt-out" of the questions. Internal consistency and reliabilities have been reported above .80 for all scales and the measure has demonstrated test-retest reliability.

Chronic Graft-versus-Host Disease Symptom Checklist

The c-GHVD symptom checklist is a 30 item, 7-subscale symptom self-report scale where transplant recipients evaluate symptom impact of chronic Graft-versus-host disease over the last month. Reliability and validity testing demonstrate that this measure is more specific and sensitive for identifying chronic versus host disease impact when compared to other quality of life measures such as the SF-36 and FACT-BMT. It has demonstrated an internal consistency (Cronbach's α 0.79-0.85), test-re-test reliability on all subscales (0.74-0.83) (Lee et al., 2002).

Completion of all measures is expected to take approximately 15-20 minutes. The questionnaires will be sent to recipients for completion and return to NIH if the subject is unable or not scheduled to come to the Clinical Center for in-person follow-up.

8.0 DONOR EVALUATION, STEM CELL COLLECTION AND PROCESSING PLAN

8.1 Pre-Study Consult and Evaluation (The following tests may be done under the 97-H-0041 protocol)

- History and physical examination
- Chest X-ray in donors with underlying pulmonary disease or history of smoking
- HLA typing class I (A, B, Cw) and Class II (DR,DQ) with high resolution DR, DQ High Resolution HLA, -A, -B, -C, -DR, -DQ typing
- Low resolution class I A, B, C and class II DR,DQ and High resolution class II DR/DQ
- Antibody screen Class I and Class II (A, B, C; DRB1; DQB1); HLA sequence base typing
- Confirmatory typing when patient arrived at NIH, high resolution DR/DQ and sequence base A, B, C
- Confirm HLA compatibility with recipient ($\geq$3/6 and $\leq$ 5/6 HLA match)
- Rule out sickling hemoglobinopathies including HbSS, HbSC by history and peripheral blood smear--donors with HbAS are acceptable
- HbsAg, anti-HBc, anti-HCV, anti-HIV, anti-HTLV, anti-CMV, RPR, West Nile virus NAT (nucleic acid test), T.cruzi (Chagas), VZV serology--this screening for infectious disease markers will be repeated if more than 30 days have elapsed since baseline screening
- Red blood cell ABO group, Rh type, antibody screen
- CBC with differential~~,~~
- Coagulation screen~~,~~
- Acute, mineral, hepatic, uric acid, LDH, protein, cpk
- pregnancy test (females of childbearing potential)
- Profile STR
- Fit to donate: Orientation - visit to Department of Transfusion Medicine - inspection of veins to determine the need for a central line for apheresis
- Consent to undergo filgrastim (G-CSF) mobilization and donate leukocytes
- KIR genotyping
- If there is a suspicion of familial BMFS in the recipient, then the haplo donor must have undergone genetic testing for genes associated with BMFS – performed at a CLIA-certified laboratory, prior to enrolling in this protocol (cohort 1 subjects only). Testing of genes related to inherited BMFS - telomere length, TERC and TERT mutations to be conducted on protocol 04-H-0012 or elsewhere prior to enrolling on 04-H-0012 to be eligible for this protocol
- Research blood for laboratory studies

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 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 pediatric provider (pediatrician, pediatric nurse practitioner or pediatric physician's assistant) will be provided who is separate from the transplant team and is charged to consider the health and welfare of the minor donor. This practitioner will serve as the donor's health care provider and advocate during the minor's participation on the clinical trial.

8.3 Apheresis of Haplo Donor for Lymphocytes (optional, ages 18 and older only)

Collection of lymphocytes for haplo donor T cells (or lymphocytes) will be collected from the allograft, after CD 34 selection, for cryopreservation for studies of donor T cell alloresponses and post-transplant studies.

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 stem cell mobilization with filgrastim (G-CSF, Neupogen®, Amgen®) as an outpatient. Filgrastim (G-CSF) will be administered based on body weight (see below) for 5-7 days, subcutaneously. The doses for days 1-4 may be given at any time of day, but the doses for days 5, and 6, and if necessary day 7 must be given very early in the morning, prior to apheresis. Predictable side effects of filgrastim (G-CSF), including headache, bone pain, and myalgia, will be treated with acetaminophen or ibuprofen. Prophylactic treatment of these side effects with the same medications may be elected. Other side effects will be evaluated and treated accordingly.

8.4.1 Minor donor mobilization with G-CSF (≤ 18 years old)

Filgrastim (G-CSF) will be administered according to a vial based algorithm to reduce wastage, improve donor compliance, and optimize CD34 yields.

Donor Wt	Total filgrastim (G-CSF) Dose (range)
15-18.9 kg	10 mcg/kg/dose
19-30.9 kg	300 mcg (10.0 to 15.8 mcg/kg)
31-37.9 kg	480 mcg (10.0 to 13.0 mcg/kg)
38 – 48.9 kg	600 mcg (12.5 to 15.8 mcg/kg)
49 – 56.9 kg	780 mcg (13.9 to 15.9 mcg/kg)
57 – 60.9 kg	900 mcg (15.0 to 15.8 mcg/kg)
61 – 67.9 kg	960 mcg (14.3 to 15.7 mcg/kg)
68 – 108.9 kg	1080 mcg (10.0 to 15.9 mcg/kg)
> 109 kg	1200 mcg (11.0 or less)

8.4.2 Adult donor mobilization with G-CSF (> 18 years old) – per Supportive Care Guidelines

Dose algorithms

Two dosing algorithms are recommended for use in the Clinical Center. The **standard dose and higher dose algorithm. The higher dose algorithm** is intended for use in donors whose components will undergo further processing. As this study involves grafts that will undergo cell processing, the higher dose algorithm will be used for all donors enrolled on this protocol. The total dose is capped at 1200 mcg/kg/day.

I. Higher-Dose Filgrastim Algorithm

Donor Weight	**Total Daily Filgrastim**	**Dose (range)**
38 – 48.9 kg	600 mcg	(12.5 to 15.8 mcg/kg)
49 – 56.9 kg	780 mcg	(13.9 to 15.9 mcg/kg)

57 – 60.9 kg	900 mcg	(15.0 to 15.8 mcg/kg)
61 – 67.9 kg	960 mcg	(14.3 to 15.7 mcg/kg)
68 – 108.9 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 & Processing

PBSC apheresis will be done on day 5, and if necessary on day 6 and day 7 of filgrastim (G-CSF), i.e., after the 5th, and possibly the 6th, and if necessary the 7th day 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 in the femoral position, using the Baxter CS3000Plus or the Cobe Spectra. 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 G-CSF and the CD34 cell dose needed, based on kilogram weight of recipient. This will range from 5 to 35 liters processed per day for 1 to 3 days, not to exceed a total of 75 liters over 3 days. In pediatric subjects, defined as less than 40 kg, a maximum of 8 blood volumes will be processed per day, for up to 1 to 3 days. The second and third day apheresis collection will not be required if the CD34 dose is 3 $x10^6$/kg or more after the first day of collection.

Anticoagulation will be accomplished with acid citrate dextrose (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.

If the minimum CD34+ cell dose is not achieved, a third apheresis may be performed (after an additional [7^{th}] dose of filgrastim [G-CSF]), if it is predicted that the minimum CD34 cell target can be achieved in this way.

If the minimum CD34+ cell dose from the haploidentical donor has not been achieved after one cycle of mobilization, a 2^{nd} mobilization with up to two full apheresis collections to achieve the minimum CD34+ cell dose may be performed after an interval of at least 10 days or an alternative related haploidentical donor may be selected to undergo mobilization.

Stem cell products will be cryopreserved after each collection and stored until transfusion into the recipient (see NIH Clinical Center, Department of Transfusion Medicine, 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.

8.6 Ex Vivo Processing of Peripheral Blood Stem Cells (PBSC) and Lymphocytes

In general, the PBSC processing goals are to maximize CD34+ cell content and deplete CD3+ lymphocytes using the CD34-selection system from Miltenyi (see section 15). 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 1 x 10^4/kg.

The erythrocyte content of the apheresis product is approximately 1.0 ml of packed RBCs per liter processed. Although the typical RBC content of a 25-liter PBPC product would be about 25 ml, processing by positive selection will typically result in a product with less than 1.0 ml of

RBCs. Donor plasma will also be eliminated by the processing procedures. Therefore, even if there is ABO or other RBC incompatibility between donor and recipient, no specific processing or precautions will need to be taken with the PBSC product.

8.7 Sterility and Viability Testing

Sterility testing is done prior to cryopreservation. For the PBSC product, the results should be available before the final product is given to the recipient but the infusion date is variable and depends on the recipient's transplantation schedule. If the final result (no growth at 14 days) is not available before the final product is given to the recipient, the principal investigator (PI) is notified. If a culture is positive at any time during the incubation period, the microbiology testing laboratory performs a gram stain on the liquid media and notifies the cell therapy lab of the preliminary morphologic finding. The cell therapy lab will immediately notify the PI. Concurrently, the testing lab will plate the liquid media onto solid media for organism identification and sensitivity testing. These results are usually available within 48-72 hours. The results are immediately reported to the PI by the cell therapy lab personnel.

Products released for infusion into the recipient are in full compliance with 21 CFR subpart C (1271.45-1271.90) which sets out the requirements for determining donor eligibility.

Viability testing is performed with 7-AAD via flow cytometry.

8.8 Cryopreservation, Thawing and Quality Systems for the Stem Cell Product

Stem cell products will be cryopreserved after each collection and stored until transfusion into the recipient.

Post CD34 enrichment, PBSC products are usually cryopreserved in vials; rarely in 25ml bags. The container used at cryopreservation depends on the cell content of the product. Below is a table modified from DTM cryopreservation and thaw SOPs (DTM-SOP-5101 and 5102, respectively) which details the volumes and range of cell concentrations at cryopreservation and at infusion. Products cryopreserved in vials are transferred to another container for infusion. Transfers are performed in a biologic safety cabinet. Since the final product for infusion is transferred from the vial used to cryopreserve the product to another container, another sterility sample will be obtained. The sample for testing is obtained from rinsing the original cryopreservation container with the same diluent as used to dilute the final product. 50 ml bags will not be used for this protocol, but are included here for completeness.

Cryo container	Minimum concentration at cryo	Maximum concentration at cryo	Maximum vol at cryo	Max TNC/ container	Volume at infusion	Maximum concentration of final product
5 ml vial	2.5e6/ml	100e6/ml	4.5ml	450e6	30 ml	1.5e7/ml
Bag, 25ml	20e6/ml	300e6/ml	25 ml	7.5e9	31 ml	2.4e8/ml
Bag, 50ml	20e6/ml	300e6/ml	50 ml	1.5e10	62 ml	2.4e8/ml

TNC=total nucleated cell count

All products will be prepared for infusion by standard operating procedures of the DTM Cell Processing Section. Detailed information is 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 a detailed description of the

DTM's quality assurance plan, 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, environmental impact and good laboratory practice statements.

8.9 Donor Monitoring and Follow-up Plan

Donors will be contacted by our clinical staff within 48 hours after their cell collection. Donors will remain on study for one year following enrollment. In the event that a recipient needs a stem cell boost following transplant, the donor may re-sign onto the protocol if the one-year time frame has elapsed.

9.0 UMBILICAL CORD BLOOD PROCESSING

Pre-cryopreservation: Only licensed cord blood units will be used or if a licensed cord blood unit is not available, umbilical cord blood (UCB) units will be obtained through the NMDP multicenter access and distribution protocol for unlicensed cyropreserved cord blood units. Cord blood units will be collected and cryopreserved according to the Cord Blood Bank Participation Criteria, including Policies and Procedures stated therein, and the NMDP requirements for product release. Donor eligibility determination is performed according to NMDP standards and/or FACT-Netcord guidelines in the case of cord blood units. The NMDP standards are set by a board of NMDP network representatives including cord blood banks, transplant centers, donor and recruitment centers, and follow the most recent FDA eligibility guidelines.

The table below details the infectious disease marker testing and acceptance criteria (suitability criteria for cord blood units):

Test	**NMDP action**
HBsAg	Accept non-reactive
Anti-HBc	Accept non-reactive
Anti-HCV	Accept non-reactive
Anti-HIV1/2	Accept non-reactive
HIV p24Ag	Performed if HIV-1 NAT testing is not performed Accept non-reactive
HIV-1 and HCV	Accept non-reactive
Anti-HTLV I/II	Accept non-reactive
Anti-CMV IgG or total	Accept non-reactive Accept reactive
Serologic syphilis	Accept non-reactive

Ordering procedures: Jennifer Wilder, the NIH Certified Hematopoietic Umbilical Cord Transplant Coordinator will conduct the search and arrange for procurement of unrelated umbilical cord product for this protocol. Once a suitable cord blood product is identified, shipment of the cord blood unit will not be requested until the DTM confirms that the G-CSF mobilized T-cell depleted graft collected from the haploidentical donor contains a suitable number of CD34+ cells ($\geq 2 \times 10^6$/kg) and CD3+ T-cells ($<1 \times 10^4$ CD3+ cells/ kg) (i.e. technical failure of the depletion process has not occurred). Once CD34+ cell content is confirmed, the cord blood unit will be shipped to the Department of Transfusion Medicine Cell Processing Section (CPS) per NMDP procedures, in dry shippers.

Storage of cord blood units: Upon arrival, the UCB units will be transferred to LN2 storage in CPS until date of infusion. Prior to infusion, the PI, in consultation with CPS, will evaluate the RBC content, if ABO incompatible, to determine whether washing the UCB to remove excess RBC is warranted. Whether the UCB units are thawed and released for infusion with or without washing, UCB units will be stored, processed and released for infusion per DTM CPS procedures (see NIH DTM SOP 5107 [product receipt] and NIH DTM SOP 5102 [thaw and infusion]).

Post-cryopreservation*:* Each product is thawed and prepared for immediate infusion. Sterility and viability testing is done post thaw as is a progenitor cell assay (CFU) and a complete cell count. The cord units will be infused with a goal viability of >= 70%. Cord units with lower than this viability will still be infused and we will track the outcome of these poorer viability units and report them in our annual report. Viability is performed with 7-AAD via flow cytometry. The sample is obtained post thaw, post dilution.

Laboratory staff coordinates closely with the nursing staff administering the product such that the product is thawed and delivered to the recipient's bedside for immediate infusion. Product is not stored post thaw.

10.0 MANAGEMENT OF COMPLICATIONS

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

10.1 Acute GVHD (See PBSCT Supportive Care Guidelines**)**

10.2 Chronic GVHD (See also PBSCT Supportive Care Guidelines**)**

- Tacrolimus and/or cyclosporine at standard dose.
- Prednisone dosed according to severity.
- Change to alternate day steroid and Tacrolimus/cyclosporine therapy when response is established.
- Non-responding subjects may be treated with other standard of care therapies including but not limited to PUVA, mycophenolate, azathioprine, photopheresis, daclizumab, infliximab, rituximab, or thalidomide at the discretion of the attending physician.

10.3 CMV Reactivation (see section 7.3 Infection Prophylaxis and Treatment)

10.4 EBV reactivation (see section 7.3 Infection Prophylaxis and Treatment)

10.5 Graft Failure/Relapse of Marrow Aplasia

Recipients who fail to achieve a neutrophil count of ≥500 cells/µl by day 42 post transplant will be deemed to have primary graft failure. Subjects with primary graft failure will be treated with supportive care (transfusions, growth factors, etc) when clinically indicated. If the subject is found to have one or more additional HLA compatible (i.e. ≥ 4/6) cord blood units, and remains neutropenic, they will be offered co-enrollment onto the Branch's standard of care protocol where they may be treated with a subsequent single or double cord unit transplant using a conventional transplant approach. Subjects who are not a candidate for a salvage cord blood transplant will be treated with supportive care and may be referred back to their referring physician if it is in the best interest of the subject. 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.

Transplant recipients with secondary graft failure may be treated with a stem cell boost. Secondary graft failure is defined as the development of sustained cytopenia and/ or continued requirement for platelet or red blood cell transfusions after initial successful primary engraftment. In this setting, a boost of hematopoietic stem cells from the original stem cell donor may be subsequently administered to the recipient. This process will involve the infusion of $CD34^+$ cells that are collected from the original haploidentical stem cell donor following conventional GCSF mobilization that are CD34 selected using the Miltenyi CliniMACS system. In the event the transplant recipient is unable to return to the Clinical Center for this infusion (i.e., due to hospitalization related to illness) these cells may be shipped by the Department of Transfusion Medicine to a hospital with a transfusion service for infusion consistent with their Human Subjects Protection Policies.

10.6 Pulmonary Engraftment Syndrome

Subjects who develop pulmonary engraftment syndrome (most likely 10-25 days post transplant) will be treated with steroids.

11.0 ANCILLARY LABORATORY RESEARCH STUDIES

During the course of study participation, specimens will be collected for correlative studies.

11.1 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 NIH Intramural IRB and are listed in the Appendix of the protocol.

Hemorrhagic cystitis study: We will share the following de-identified research samples with Dr Christopher Buck's laboratory at NCI: blood, urine, serum, plasma, CSF, Lung lavage fluid. His lab will perform deep sequencing on DNA extracted from BKV viral particles using the Illumina Nextera/MiSeq platform. BKV neutralization serology will also be performed.

Hepatitis E research study: We will share de-identified plasma samples with Dr A. Gretchen Buckler's laboratory at DTM/Clinical Center. Her lab will test them for hepatitis E RNA and, if they find positives, will work towards setting up a prospective trial to determine if future patients show evidence of contracting hepatitis E through transfusion using linked donor-recipient pairs.

Etiology of post-transplant syndromes: We will share de-identified blood plasma and/or serum with Dr. Waldman at NIDDK. Dr. Waldman will use blood plasma and or serum to conduct studies on the etiology of post-transplant syndromes that cause renal failure including nephrotic syndrome, TMA, and glomerulonephritis.

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 not be sent outside NIH without IRB

notification and an executed MTA. In accordance with the NHLBI Biospecimen Inventory system (BSI) policy, the specimens are inventoried in BSI.

Storage: Research samples will be stored with identifiers in the secure laboratory of the principal investigator.

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.

11.2 Research Sample Collection

11.2.1 *Recipient peripheral blood samples:* 60 ml peripheral blood will be collected at days 15, 30, 42, 60, 100 (+/- 7 days for all) and then at scheduled annual follow-up visits for chimerism analysis lymphocyte subset analysis.

Subjects will be offered co-enrollment on protocol 07-I-0183: The collection of research samples and data for repository from unrelated hematopoietic stem cell transplantation recipients for the National Marrow Donor Program. If consented to participate on this ancillary study, one ACD (acid citrate dextrose) anti-coagulated blood-20 ml drawn in a yellow top tube - will be sent to the NMDP research sample repository. A MTA for these samples has been executed. In pediatric recipients, the amount drawn will be 1-20 ml, not to exceed 3ml/kg (the NMDP has no minimum blood sample size from a pediatric subject).

11.2.2 *Recipient apheresis:* One apheresis collection of approximately 10^{10} leukocytes in a volume of 200 ml, pre-transplant.

11.2.3 *Donor blood sample:* Cells obtained during the apheresis procedures (stem cells and lymphocytes) additional to those required for the transplant recipient will be used for research studies.

11.2.4 *Recipient bone marrow samples:* An extra volume (up to 25 ml) of bone marrow aspirate will be collected for research studies at the pre transplant evaluation and when collecting for monitoring 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.

11.2.5 *Pulmonary function tests*

Pulmonary status at baseline, day 30 and after day 100 will be analyzed to see if reduced intensity conditioning (RIC) effects lung function before day 100 or if the decline in lung function is only seen later, consistent with bronchiolitis obliterans syndrome (BOS). Pulmonary status will be assessed in all subjects except for those under the age of 10 where prior attempts have failed because of the subject's inability to perform the pulmonary function test.

12.0 BIOSTATISTICAL CONSIDERATIONS

12.1 Primary Endpoint

The primary endpoint is donor engraftment by day 42 +/- 3 days (defined as an ANC of >500) from either the haplo donor, the cord, or both combined.

12.2 Secondary Endpoints

Secondary endpoints will include:

- ANC recovery rate (ANC ≥ 500 cells/μl) at day 22, 100 day (+/- 3 days) and 6 months (+/- 2 weeks)
- treatment related mortality (TRM)
- standard transplant outcome variables such as non-hematologic toxicity, incidence and severity of acute and chronic GVHD, and relapse of disease.

Health related quality of life will also be assessed as secondary outcome measures. The following parameters will be recorded:

- CD34 + cell dose, CD3 + cell dose
- Degrees of cord blood, related haplo-CD34+ cell donor and recipient chimerism in T-lymphocyte, NK cell, and myeloid lineages assessed by PCR of short tandem repeats (STRs)
- Neutrophil recovery (days to neutrophil count of 0.5 x 10^9/l)
- Days to an UCB neutrophil count of 0.5 x 10^9/l
- Platelet recovery (days to platelet count of 20 x 10^9, days to transfusion independence)
- Red cell recovery (days to transfusion independence)
- Incidence and severity of acute and chronic GVHD (the DSMB will monitor for evidence of excessive GVHD)
- Graft failure (loss of the graft as measured by chimerism)
- Cumulative incidence of late graft failure
- Time to transfusion independence
- Non-hematological effects attributable to the preparative regimen
- Disease progression or relapse
- Transplant related mortality before and after day 100 and day 200
- Disease-free survival and overall survival.
- Health related quality of life measurements post-transplant

12.3 Sample Size

We require engraftment by day 42 in at least 80% of recipients and wish to insure that engraftment is not as low as 60%. Let p denote the probability of engraftment by day 42. We will test the null hypothesis that p is less than or equal to 0.6 versus the alternative hypothesis that p is at least 0.8. We choose a two-stage minimax design [102] which allows early stopping if too few recipients engraft and minimizes the maximum sample size subject to alpha = 0.05 and power = 0.80.

In the first stage we will enter (up to) 13 recipients. If 8 or fewer have engraftment by day 42, we stop early because the procedure is not successful enough. This implies that we must have at least 9 of the first 13 recipients with engraftment by day 42. If at least 9 recipients have engraftment by day 42, we will enter an additional 22 recipients. If 25 or fewer of the total of 35 recipients have engraftment by day 42, we consider the procedure unsuccessful (rejecting the alternative

hypothesis), but if at least 26 of the 35 recipients have engraftment by day 42, then we consider the procedure successful.

To account for the possibility of non-treatment related deaths before day 42, an additional 5 subjects may be enrolled to replace those subjects that are non-evaluable for the study's primary endpoint.

12.4 Methods of Statistical Analysis

Although the primary endpoint for this protocol is persistent engraftment by day 42, subjects will be followed up for 60 months on protocol and then contacted indefinitely to monitor the incidence of chronic GVHD, long-term disease-free and overall survival.

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 or competing risk methods, as appropriate, and 95% confidence intervals for the median time to events will be calculated.

12.5 Interim Analysis and Stopping Rules

Interim analysis for efficacy.
An interim subgroup analysis is planned when at least 50% of recipients are evaluable for primary endpoint. A modified Haybittle-Peto procedure with Bonferroni adjustment will be used: we would require a conservative p-value <0.001 to declare significance and reject the null hypothesis of primary endpoint at the interim analysis. We would not stop the trial based on early evidence of clinical benefit at the interim subgroup analysis, and we would continue enrollment to the original planned sample size. At the final analysis we would require p<0.049 to declare significant improvement in the primary endpoint.

Stopping rule for early death: If there are 5 treatment related deaths (TRM) prior to day 42, we will stop the trial early. Early deaths determined to be related to treatment will be included in the stopping rules for safety. If we are convinced that a death before day 42 is unrelated to treatment, we will ask the IRB to review the data and permit continuation if deemed appropriate.

Stopping rule for futility: In the first stage, if 5 recipients fail to have engraftment by day 42, we will stop the trial early (even before all 13 recipients are enrolled). If at least 9 recipients have engraftment by day 42, we will start the second stage. Then if there are a total of 10 or more recipients who fail to have engraftment by day 42 in the first and second stages, we stop the trial early (even before additional 22 recipients are enrolled in the second stage).

***Stopping rules for safety*:** We wish to assure that this procedure has less than 50% day 200 TRM. We will use a Bayesian stopping rule. The stopping boundary is reached if the Bayesian posterior probability that the true probability of 200 day TRM exceeds 0.45 is at least 90%. We take our prior distribution to be a beta distribution so that our prior clinical opinion is worth 20% of the weight we will place on the new data. This gives prior parameters alpha = 4.05, beta = 4.95.We will begin monitoring 200 day TRM when 5 recipients are evaluable for 200 day TRM.

The following table summarizes the threshold numbers for the boundary, which would lead to a recommendation to stop the study due to excess day 200 TRM.

Number of subjects	Stop if the number who have developed 200 day TRM reaches
Up to 5	5
6-7	6
8-9	7
10-11	8
12-13	9
14-15	10
16-17	11
18-19	12
20-21	13
22-23	14
24-25	15
26-27	16
28-29	17
30-31	18
32-33	19
34-35	20

We conducted a simulation to evaluate this stopping rule. We generated a study with 35 independent Bernoulli trials, with probability p for 200 day TRM and determined whether the study was stopped. We repeated the simulations 100,000 times. When the probability of 200 day TRM was 0.4, the probability of stopping the study was 0.10. When the probability of 200 day TRM was 0.5, the probability of stopping the study was 0.40. When the probability of 200 day TRM was 0.6, the probability of stopping the study was 0.78. When the probability of 200 day TRM was 0.7, the probability of stopping the study was 0.98. Hence we believe that this stopping rule has satisfactory statistical properties.

In addition, the DSMB will evaluate all treatment related serious adverse events (TRSAEs). The DSMB may recommend early study termination of the trial if other unforeseen adverse events necessitate this decision.

12.6 Off Study Criteria

12.6.1 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, the recipient will be followed in the safety analysis.

Without compromising their participation in the treatment protocol recipient and donor may at any time withdraw from participation in the ancillary laboratory research aspects of the protocol.

12.6.2 Withdrawal by principal investigator decision

Transplant recipients who

- experience disease progression or relapse
- fail to achieve hematological recovery (graft failure) or

- experience a significant decline in performance status which negates further treatment on protocol

will be offered co-enrollment onto the standard of care protocol, evaluated for participation on another primary treatment protocol or referred back to their home physician depending on what is in the best interest of the recipient. Data collection to include outcome and survival will continue, however complications following alternative therapies will not be reported on this protocol.

12.6.3 Completion of the study

Following the completion of 5 years of follow up care the recipient will go off study and be referred back to their primary care physician. Yearly communication with the recipient will be continued since outcome data for the recipient's life span is required by the NMDP. Donors will remain on study for one year following enrollment. In the event that a recipient needs a stem cell boost following transplant, the donor may re-sign onto the protocol if the one year time frame has elapsed.

13.0 DATA AND SAFETY MONITORING

13.1 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 12.5).

***NIH Intramural IRB*.** Accrual and safety data will be monitored reviewed annually by the Institutional Review Board (IRB). Prior to implementation of this study, the protocol and the proposed subject consent and assent forms will be reviewed and approved by the properly constituted Institutional Review Board (IRB) operating according to the 45 CFR 46 Code of Federal Regulations. This committee must approve all amendments to the protocol or informed consent, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects.

***DSMB*:** The NHLBI Data Safety and Monitoring Board will review the protocol at six or twelve month intervals. 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.

Monitoring: As per ICH-GCP 5.18 and FDA 21 CFR 312.50, 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 and 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, FDA, the site monitors, and the NHLBI staff for confirmation of the study data.

FDA (IND 13500): An annual progress report, amendments to the protocol, and changes in the status of the protocol will be forwarded to FDA to:

Candace Jarvis
Center for Biologics Evaluation and Research (CBER)
Office of Cellular and Gene Therapy
Document Control Center, HFM-99, Suite 200N
1401 Rockville Pike
Rockville, MD 20852-1448
Phone: 301-827-5357

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

Tara Clark, General Manager US Clinical Operations
and Norman Pilon, PhD
Miltenyi Biotec, Inc.
85 Hamilton Street,Cambridge, MA 02139.
Phone (617) 218-0060

13.2 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.

Suspected adverse reaction: Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

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.

Unexpected adverse reaction: An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

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.

Non-compliance: The failure to comply with applicable NIH HRPP policies, IRB requirements, or regulatory requirements for the protection of human research. Noncompliance may be further characterized as:

1. Serious non-compliance: Non-compliance that:
   a. Increases risks, or causes harm, to participants.
   b. Decreases potential benefits to participants.
   c. Compromises the integrity of the NIH HRPP.
   d. Invalidates the study data.

2. **Continuing non-compliance:** Non-compliance that is recurring. An example may be a pattern of non-compliance that suggests a likelihood that, absent an intervention, non-compliance will continue. Continuing noncompliance could also include a failure to respond to IRB requests to resolve previous allegations of non-compliance.

3. **Minor (non-serious) non-compliance:** Non-compliance that, is neither serious nor continuing.

13.2.1 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 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.

13.3 Adverse Events –Transplant Recipients

13.3.1 Adverse Event Reporting and Collection

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 exceptions listed below. The AE reporting period for this study begins when the patient starts the preparative regimen, except for AEs at least possibly related to a study procedure performed prior to the start of the preparative regimen (i.e., apheresis). AEs will be collected until completion of the 1 year follow up. After that time, only SAEs that are at least possibly related to the transplant will be recorded and reported to the IRB. The AEs will be attributed (unrelated, unlikely, possibly, probably or definitely) to study medication and graded by severity utilizing NCI Common Terminology for Adverse Events (CTCAE) 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.

Events not included in the CTCAE will be graded as follows:

Grade 1: Mild: discomfort present with no disruption of daily activity, no treatment required beyond prophylaxis
Grade 2: Moderate: discomfort present with some disruption of daily activity, require treatment
Grade 3: Severe: discomfort that interrupts normal daily activity, not responding to first line treatment
Grade 4: Life-threatening: discomfort that represents immediate risk of death

The following are expected outcomes for the transplant recipient and will be documented in the subject's medical record, but will not be collected or reported to IRB unless they meet the criteria for an SAE and/or UP:

- 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: cataracts, infertility, growth impairment, hypothyroidism, and dental caries
- 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
- 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

The following are expected outcomes related to cord blood infusion and will be documented in the subject's medical record, but will not be collected or reported to the IRB unless they meet the criteria for an SAE and/or UP:

- Changes in heart rate or rhythm
- Changes in blood pressure
- Fever, chills, sweats
- Nausea, vomiting, diarrhea, abdominal cramping, fluid overload
- Headache
- Dyspnea
- Hemoglobinuria
- Allergic reaction

The following are expected outcomes that will be collected and 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 and/or UP:

- Acute graft-versus-host disease
- Chronic graft-versus-host disease
- Graft failure/graft rejection
- Veno-occlusive disease

- Hemorrhagic cystitis
- Nephrotic syndrome
- Cytomegalovirus reactivation or disease
- EBV reactivation or disease
- Invasive fungal infections
- Disease relapse or progression
- Secondary hematologic and/or solid tumor malignancy
- Pre-malignant conditions such as myelodysplastic syndrome
- Hemophagocytic Lymphohistiocytosis (HLH)

13.4 Adverse Events - Donors

The following are expected outcomes for the donor that will not be collected or reported to the IRB unless they meet the criteria of an UP:

- 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

The following are expected outcomes for the donor that will be collected and 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 UP:

- Ischemic chest pain during filgrastim (G-CSF) administration
- Splenic enlargement
- Cutaneous vasculitis
- Bone pain, muscle aches or headaches no controlled with non-narcotic analgesics

13.5 Reporting of SAEs and UPs

All serious adverse events will be reported to
Richard Childs, M.D.; Principal Investigator
Bldg 10, CRC, Room CRC 3-5332
Phone: 301-594-8008

FDA (IND 13,500) SAEs that are **unexpected and suspected** (original and 2 copies) will also be forwarded as soon as possible and no later than seven calendar days in the case of death or life-threatening serious adverse events or within fifteen calendar days after the occurrence of all other forms of serious adverse events to the FDA. A summary of all SAEs will be included with the annual progress report to the FDA.

Candace Jarvis
Center for Biologics Evaluation and Research (CBER)
Office of Cellular and Gene Therapy
Document Control Center, HFM-99, Suite 200N
1401 Rockville Pike
Rockville, MD 20852-1448

Phone: (301)827-5357

DSMB: Reports of serious adverse events that are **unexpected and suspected** will also be forwarded as soon as possible and no later than seven (7) days in the case of death or life-threatening serious adverse events or within fifteen (15) days after the occurrence of all other forms of serious adverse events to the Data and Safety Monitoring Board (DSMB). A summary of all SAEs will be included for review semiannually by the DSMB.

If the serious adverse event is thought to be due to the experimental component of the protocol, accession to the protocol will be stopped until a full discussion with the IRB has been held.

Miltenyi Biotec, Inc.: Reports of serious adverse events will be forwarded to Miltenyi Biotec, Inc. to

Tara Clark, General Manager US Clinical Operations
and Norman Pilon, PhD
Miltenyi Biotec, Inc.
85 Hamilton Street, Cambridge, MA 02139.
Phone (617) 218-0060

13.6 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. The principal investigator, associate investigators, NHLBI fellows, research nurses and/or a contracted data manager will assist with the data management efforts. Data will be abstracted from Clinical Center progress notes as well as from progress notes forwarded from home physician. Laboratory data from NIH will be imported electronically from CRIS into an in-house clinical trial database. Laboratory values from referring home physicians will be entered into the system.

Research data will be prospectively collected by authorized Investigator personnel and entered into the NHLBI Medidata Rave Database. Medidata Rave is a web-based, 21 CFR 11 compliant, database which will consist of the study specific set of electronic CRFs (e-CRFs) used for capturing, managing and reporting clinical research data.

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

CIBMTR: Subjects will be offered co-enrollment on protocol 07-I-0183: The collection of research samples and data for repository from unrelated hematopoietic stem cell transplantation recipients for the national marrow donor program. If consented to participate on this ancillary study the *National Marrow Donor program (NMDP)* will be forwarded transplant outcome data. Data reporting requirements for the NMDP Coordinating Center include: Baseline confirmatory data, pre-conditioning, 100 day, 6 month, 1 year and annually post-transplant outcome data for the recipient's life span. Data reporting requirements for the NMDP Donor Center include: 30 day, 6 months and 1 year post transplant updates.

For the purposes of quality assurance (i.e. accreditation of the NHLBI Transplant program), de-identified, anonymous data will be released to the Center for International Blood and Marrow Transplant Research (CIBMTR) according to federally mandated policies and procedures.

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.

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

Publication policy: Given the research mandate of the NIH, subject 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 (OHSR).

14.0 HUMAN SUBJECT PROTECTIONS

14.1 Rational for Subject Selection

Aplastic anemia (AA): The study will be open to all AA subjects who satisfy the inclusion criteria and provide an informed consent to the protocol. No subjects will be excluded from participation based on gender, race or ethnicity. Epidemiologic studies suggest that the gender will be approximately evenly split between male and females, and that 90% of the subjects will be Caucasian.

Myelodysplastic syndrome (MDS): The NHLBI has a robust source of subjects with bone marrow failure and we do not anticipate recruitment to be problematic. Since 1997, we have screened over 400 subjects with MDS (about 30/year).

Recruitment efforts: The study will be listed on ClinicalTrials.gov, the Clinical Center research studies website, The Aplastic Anemia Foundation, and the National Heart, Lung and Blood Institute subject recruitment website. If recruitment goals are not met, a recruitment plan will be developed by the Clinical Center Office of Patient Recruitment.

Competition between Branch protocols: There are no competing branch protocols for this subject population.

Reimbursement for protocol participation, travel, food, and lodging will be consistent with NIH guidelines. In determining reimbursement, the following factors are considered applicable to this protocol: the patients are diagnosed with a rare disease; the patient population is sick; the protocol offers the potential for direct benefit; the protocol regimen is demanding; and in order to complete accrual in a reasonable timeframe a geographically dispersed participant population is required.

Payment for participation: $0

14.2 Participation of Children

14.2.1 As recipients:

Although the time to engraftment is quicker in younger children receiving cord blood transplants compared to adults, neutrophil recovery is still delayed compared to the use of a peripheral blood stem cell transplant. For patients with aplastic anemia who have had prior opportunistic infections or who have an active infection at transplant, expediting neutrophil recovery by co-transplanting haploidentical stem cells could potentially reduce the risk of infectious mortality associated with conventional cord transplantation. Furthermore, graft rejection occurs frequently in children with aplastic anemia undergoing conventional cord blood transplantation. The use of haploidentical stem cells may also serve as a backup stem cell source should the graft be rejected, which potentially could prevent rejection related mortality.

14.2.2 As donors:

Allogeneic bone marrow (and peripheral blood) transplantation (BMT) provides an option for cure for aplastic anemia and is therefore is considered an accepted standard clinical intervention for this disease. The donor would be donating stem cells to his/her family member regardless of the objectives of this research protocol. We are however excluding from participation as donors, children who weigh $\leq$18 kg and are < 6 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 almost always needed.

19 to 25 kg: A central line is required. Donors may or may not need a central line (at the discretion of the apheresis department). With concurrent magnesium and calcium infusion, children may be safely anti-coagulated with citrate.

On sister protocol 99-H-0050, 5 of 10 (50%) of minor donors (4/15/2009) required femoral lines, three of which (ages 6, 7, and 10) required conscious sedation.

14.2.3 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.

14.3 Hazards and Discomforts

14.3.1 The recipient

Related to venous access placement: Intravenous line placement carries a small risk of bleeding, bruising or pain and a very low risk of accidental injury to the adjacent artery and nerve. Some participants may experience a vasovagal reaction (lightheadedness, or, rarely fainting due to temporary lowering of blood pressure).

Related to bone marrow aspiration: No major risks are involved with bone marrow aspirate and biopsy. However, a small risk of infections, pain, bleeding, and hematoma formation at the site of the aspiration exists with the procedure.

Related to chest x-ray: The risks associated with x-rays are very small. For example, one chest x-ray has the same increased risk of death (due to cancer) as smoking 1.4 cigarettes or drinking ½ liter of wine. On average, all of us receive an equivalent dose of x-rays every 30 days from nature. However, the more radiation received during a lifetime, the greater the risk of developing cancerous tumors or of inducing changes in genes. The changes in genes could cause abnormalities or disease in future offspring. We do not expect the radiation in this study to greatly increase these risks, but we do not know the exact increase in such risks.

Related to pulmonary function test: Risks are minimal, but some subjects may become lightheaded or faint during the test. The test could also trigger an asthma attack in persons with asthma.

Related to CT scans: Oral and/or intravenous contrast agents will be used and are usually well tolerated. However, some subjects will experience allergic reactions to intravenous contrast. To lower the risk of allergic reactions, low allergenic contrast agents are administered at NIH Clinical Center. In addition, subjects will be advised that approximately 2-7% of patients who receive contrast agents will experience a temporary reduction in kidney function lasting up to 2 weeks following infusion and that in rare instances, permanent renal damage can result from the use of the IV contrasting agent. Therefore, in subjects with impaired kidney function, we will not use intravenous contrast. The amount of radiation from a head CT is comparable to 8 months of natural background radiation.

Related to EKG (age $\geq$ 50 or $\geq$ 40 with risk factor): There is no pain or risk associated with having an electrocardiogram.

Related to echocardiogram (age $\geq$ 50 or $\geq$ 40 with risk factor): This test has no known side effects.

Related to MUGA scan (age $\geq$ 50 or $\geq$ 40 with risk factor): There are no known adverse events associated with a MUGA scan. The radiotracer used during the scan has a radiation dose similar to that of a CT scan (8 months of natural background radiation).

Related to cardiac stress test (age $\geq$ 50 or $\geq$ 40 with risk factor): The possible risks of this procedure are: an allergic reaction to the radioactive dye or a flushed feeling; lightheadedness or fainting from the exertion; an abnormal heart rhythm brought on by the exertion. Although very rare, it is possible that a nuclear stress test could cause a heart attack.

Related to the quality of life instruments: Subjects may feel uncomfortable answering the questions.

Related to the transplant procedure: Stem cell transplantation is a major procedure, which entails serious discomforts and hazards for the recipient. The major limitation of UCB transplantation in adults is the limited number of nucleated cells contained within the cord unit resulting in prolonged neutropenia and failure of engraftment, which contributes to infection and TRM. Full haplotype mismatched transplants have been associated with a risk of transplant-related mortality as high as 50%. The high frequency of alloreactive donor T cells in unmanipulated grafts that recognize mismatched MHC antigens results in an extremely high incidence of severe, acute GVHD. Although extensive T cell depletion prevents GVHD, the rejection rates rise steeply because the balance between competing host and donor T cells shifts in favor of the unopposed host-versus-graft reaction. Furthermore, T-cell depletion is associated with a greater risk of post-transplant viral infections, secondary Hemophagocytic

Lymphohistiocytosis (HLH), and disease relapse. Toxicities potentially associated with the infusion of the CBU include dimethyl sulfoxide (DMSO) toxicity and side effects from intact and hemolyzed red cells and may include changes in heart rate or rhythm, changes in blood pressure, fever, chills, sweats, nausea/vomiting, diarrhea, abdominal cramping, fluid overload, headache, dyspnea, hemoglobinuria, allergic reaction, acute renal failure, and in rare cases infusion reaction resulting in death.

The mortality from a cord blood or full haplotype mismatched transplants 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 recipient and donor and immediate family. The major hazards (graft failure/rejection, GVHD, infection, and disease relapse) have already been mentioned. 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.

Transplant recipients have been reported to have an increased risk of solid cancers but most studies are small and have limited ability to evaluate the interaction of host, disease, and treatment-related factors. In the largest study to date to evaluate risk factors for solid cancers, a multi-institutional cohort of 28874 allogeneic transplant recipients developed 189 solid malignancies. Overall, patients developed new solid cancers at twice the rate expected based on general population rates (observed-to-expected ratio 2.1; 95% confidence interval 1.8-2.5), with the risk increasing over time (P trend < 0.001); the risk reached 3-fold among patients followed for 15 years or more after transplantation. These findings showed that the risk of developing a non-squamous cell carcinoma (non-SCC) following conditioning radiation was highly dependent on age at exposure. Among patients irradiated at ages under 30 years, the relative risk of non-SCC was 9 times that of non-irradiated patients, while the comparable risk for older patients was 1.1 (P interaction <.01). Chronic graft-versus-host disease and male sex were the main determinants for risk of SCC. These data indicate that allogeneic transplant survivors, particularly those irradiated at young ages, face increased risks of solid cancers, supporting strategies to promote lifelong surveillance among these patients. (Blood. 2009; 113:1175-1183).

Secondary MDS/AML of donor origin is also a known but rare complication following allo-transplant [112-115]. This phenomenon is increasingly being realized given our ability to do chimerism assays to distinguish donor from patient cells.

Because of the nonmyeloablative nature of this regimen, is anticipated that recipients will be less likely to become infertile with this transplant approach compared to a conventional myeloablative transplant. Because it is possible that infertility may occur, we will discuss the possibility of sperm banking or egg retrieval with the subject before undergoing the transplant.

Related to the apheresis procedure: The apheresis procedures will be performed in accordance with standard apheresis donation policies and procedures operative in the Dept. of Transfusion Medicine 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 (if done via peripheral access)
- 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.

Related to the CliniMACS® CD34 Reagent System: Theoretical risks to the recipient could include system failure, user error, or recipient reaction to selected product components (i.e. residual amounts of unbound CliniMACs CD34 Reagent, iron dextran, or human anti-mouse antibodies). For full text see Investigator's Brochure, Version 7, dated 7/31/2011, section 6, Summary and Guidance for the Investigator.

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

Related to the common transplant related drugs and radiotherapy.
Antithymocyte globulin: Febrile reactions, allergic symptoms including rash, wheezing, anaphylaxis, cytopenias, serum sickness, nausea, vomiting, diarrhea, arthralgias, hypotension, tachycardia, bradycardia, fever, chills, myelosuppression. Several cases of a severe lung injury related to Atgam treatment have been reported. Although this side effect appears extremely rare, it is serious and can be fatal. There is no information about the mechanism or specific treatment for this condition. A few patients recovered after intensive medical support including use of a breathing machine.

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 patients treated with fludarabine.

Mycophenolate mofetil: Serious side effects include increased susceptibility to infection and possible development of lymphoma, and increased risk of pregnancy loss and congenital malformations (external ear and facial abnormalities including cleft lip and palate, and anomalies of the distal limbs, heart, esophagus, and kidney). Nausea, diarrhea, leukopenia, headache, weakness, urinary frequency, leg cramps or pain, liver function test abnormalities, infection related to immunosuppression, and skin rash. Hemorrhagic gastritis and pancreatitis have been reported rarely. Malignant neoplasms have been reported in psoriasis patients treated with oral mycophenolic acid, although a definite cause-effect relationship has not been established. Multifocal leukoencephalopathy (PML) caused by reactivated JC virus has been reported following MMF (10 confirmed and 7 possible cases). The latent virus is present in about 80 percent of adults. In response, in June 2008, a safety alert was issued and physicians were advised

that patients who present with or develop new neurologic signs or symptoms should be evaluated for PML. Neurological warning signs include: major changes in vision, unusual eye movements, loss of balance or coordination and/or disorientation or confusion. There are no known effective treatments for PML.

Prednisone: Sodium retention, hypertension, congestive heart failure in susceptible patients, hyperglycemia, increased requirements for insulin or oral hypoglycemic agents in diabetics, increased appetite, psychic disturbances, peptic ulcers, and increased risk for opportunistic infections. Long term administration of glucocorticoids may be associated with myopathy, cataracts, osteoporosis, osteonecrosis, and growth retardation in children.

Tacrolimus: Acute tacrolimus nephrotoxicity is usually manifested by a moderate decline in renal excretory function, which is readily reversible by a decrease in drug dosage. Although some degree of transient renal dysfunction may occur in patients with therapeutic levels of tacrolimus, significant renal toxicity is associated with elevated trough or steady state levels. In addition to an increase in BUN and creatinine, hyperkalemic hyperchloremic acidosis, low fractional excretion of sodium and the onset of hypertension with hypomagnesemia are seen with tacrolimus nephrotoxicity. Hypertension occurs in up to 60% of patients. Hypomagnesemia can be associated with neurologic symptoms, including seizures, cerebellar ataxia and depression. Dose-related hepatotoxicity, manifested by elevation of serum transaminases and bilirubin, has 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 filgrastim (G-CSF): (See section 14.3.2 below)

Related to the total body radiation*:* The side effects of radiation have been well described. The most common include nausea and mucositis. 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.

Related to fertility: Although the conditioning regimen uses low dose radiation and chemotherapy that usually does not compromise fertility, no data is currently available on the chances that subjects will maintain fertility after this type of transplant. Even those who become infertile after the transplant may regain fertility with time.

14.3.2 The donor

Related to filgrastim (G-CSF): The hazard to the donor is low. The discomforts 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. 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.

Patients with ongoing ischemic heart disease have been reported to have angina seemingly temporally-related to G-CSF administration and apheresis(Vij). 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.[104]

Related to central line placement: It is estimated that about 50% of the donors less than age 18 will require intravenous line placement (femoral vein); and about 50% of those minors will require conscious sedation for the line placement procedure to successfully complete apheresis. Intravenous line placement in the femoral vein using a temporary Quentin catheter carries a small risk of bleeding, bruising or pain, infection, and a very low risk of accidental injury to the adjacent artery and nerve. Some subjects may experience a vasovagal reaction (lightheadedness, or, rarely, fainting due to temporary lowering of blood pressure). Using only trained experienced staff for the procedure minimizes these risks.

In exceptional instances the donor is required to donate PBPC a third time. There is a minimal additional risk that the donation of PBPC on three successive days can increase the possibility of thrombocytopenia. Thrombocytopenia is transient and unlikely to cause clinical sequelae.

***Related to Apheresis*:** see section 14.3.1

14.4 Risks in Relation to Benefits

As of April 18, 2019, this study is now closed to new subject accrual and continues in Follow-up only. The level of risk is now minimal.

14.4.1 For adult transplant subjects

Clinically the approach is ethically acceptable because we are offering adult subjects with debilitating hematologic disorders who have failed or are not eligible for conventional treatments an alternative therapy which may be curative. The protocol aims to decrease the risk of transplant-related mortality associated with UCB transplantation, thus making more patients candidates for potentially curative therapy. The risk of death due to complication from the allogeneic BMT procedure is justified by the anticipated benefit of potentially eradicating the patient's SAA or MDS.

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).

14.4.2 For pediatric transplant subjects

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 debilitating hematologic disorders who have failed or are not eligible for conventional treatments an alternative therapy which may be curative

(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. Clinically the approach is ethically acceptable because the protocol aims to decrease the risk of transplant-related mortality associated with UCB transplantation, thus making patients candidates for potentially curative therapy

(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).

14.4.3 For adult donors:

An HLA partially matched 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 procedure, 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 of adults as a stem cell donors on this protocol involves greater than minimal risk but presents the prospect for direct benefit (45 CFR 46.102).

14.4.4 For pediatric donors:

An HLA partially matched pediatric family member may be co-enrolled on this study as a stem cell donor. The stem cell collection aspect of this protocol is not investigational. Despite the risks associated with this procedure, potential benefit does exist for the pediatric 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 and evaluation of overall health status of the pediatric participant at the time of screening.

Therefore, participation as a stem cell donor on this protocol involves greater than minimal risk but presenting the prospect of direct benefit to individual subjects (45 CRF 46.405).

14.4.5 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. Blood and bone marrow specimens for research are obtained concurrently with clinically indicated sampling. Immune cell collection will not be permitted in pediatric donors. Therefore, there is no risk associated with sample collection for research because research will only be performed on material obtained during standard research interventions.

Research specimens will be stored in the PI's laboratory. Samples will be assigned a unique code known only to the principal investigator, 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 will be conducted. Research will not include genetic testing. Therefore, there is no genetic testing associated risk.

(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 involves not greater than minimal risk (45 CFR 46.404).

14.5 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.

Minor subjects: If the subject is a minor, 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 legally recognized parent or 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.

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.

Consenting to pregnancy testing in minors of childbearing age: The subject will be informed during the assent process that for safety, we need to do a pregnancy test. The subject will also be told that if it is positive, we will counsel her and help her tell her parents. If the minor does not want to proceed, she will be advised not to sign the assent and her enrollment on this screening protocol will end.

Minor donors: 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.

Decisionally impaired: The IRB will be consulted prior to enrolling any decisionally impaired subject (donor or recipient).

Non-English speaking research participants: We anticipate enrolling non-English speaking research participants into this study. The IRB approved full consent document will be translated into the subject's native language in accordance with the Clinical MAS Policy M77-2. If there is an unexpected 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 a short form oral consent process as described in MAS Policy M77-2, 45CFR46.117(b)(2) and 21CFR50.27(b)(a). The summary that will be used is the English version of the extant IRB approved consent document.

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 subject participants. Documentation will be provided to the IRB and if necessary, the informed consent amended to reflect relevant information.

14.6 Conflict of Interest

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 are added. Copies of the initial Conflict of Interest Statements were forwarded to the Clinical Director. No initial members of the research team reported a potential conflict of interest.

This protocol may have investigators who are not NIH employees. Non- NIH investigators are expected to adhere to the Principles of the Protocol Review Guide but are not required to report their personal financial holdings to the NIH.

This protocol has no associated patents or CRADAs. A CTA between Miltenyi Biotec Incorporated and the National Heart, Lung and Blood Institute of the use of the CliniMACS CD34 and CD3 reagent cell selection systems for clinical research purposes was executed on 9/10/2007.

An MTA between the NMDP research sample repository and the NHLBI **has been executed** for the transfer of the pre transplant sample of peripheral blood.

A data transfer agreement between the CIBMTR and NHLBI for the transfer of transplant outcome data was executed in 2009.

15.0 PHARMACEUTICALS

15.1 CliniMACS® CD34 Reagent System

15.1.1 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

15.1.2 Physical, chemical and toxicological information

- CD34 Reagent description: The CliniMACS CD34 Reagent is a dark amber, nonviscous, 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 superparamagnetic.

- 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. 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 the following table.

Table 1: Summary of Toxicology of the CliniMACS CD34 Reagent	
Test	Results
Human Cryosection Cross Reactivity Study	CD34 Reagent specifically reacted with cell types known to posses 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 cageside observations were noted when escalating doses of CD34 Reagent
Irritation	No irritation
Hemocompatibility	Compatible with human blood
Sterility assay of the final container	Reproducibly sterile product

15.1.3 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.

15.1.4 Overall safety of the CliniMACS® CD34 Reagent System: The results summarized in the 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.

The BMT CTN #0303 Study entitled "A Single Arm, Multicenter Phase II Trial of Transplants of HLA-Matched, CD34+ Enriched, T-cell Depleted Peripheral Blood Stem Cells Isolated by the CliniMACS System in the Treatment of Patients with AML in First or Second Complete Remission" was performed in conjunction with the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). This multi-center, phase II study was performed to support the probable benefit of the CliniMACS® CD34 Reagent System for processing hematopoietic progenitor cells-apheresis (HPC-A) to obtain a CD34 positive cell enriched population intended to provide for hematopoietic reconstitution following a myeloablative preparative regimen without the need for additional graft-vs-host disease (GVHD) prophylaxis in patients with Acute Myeloid Leukemia (AML) in first or second morphologic complete remission.

The BMT CTN #0303 Study was designed to provide extensive T-cell Depletion (TCD) using the CliniMACS® CD34 Reagent System, to eliminate the requirement for post-transplant pharmacological GVHD prophylaxis in adult patients with AML in first or second CR undergoing a myeloablative transplant from a matched related donor. The goal was to demonstrate that the side effects of T-cell depleted allogeneic hematopoietic stem cell transplantation (HCT) would be reduced if combined with a conditioning regimen that was highly immunosuppressive and anti-leukemic. The primary objective was to achieve a disease-free survival (DFS) rate at six months post-transplant that exceeded 75%. Secondary objectives included assessments of engraftment, overall survival, EBV reactivation, transplant-related

mortality (TRM), acute and chronic GVHD, relapse and performance of the CliniMACS® CD34 Reagent System.

Forty-seven patients were enrolled and 44 transplanted at eight different centers in the BMT CTN network. The mean age was 46.3 years with 28 female and 16 male patients. Of the 37 AML CR1patients on protocol, 27% had an unfavorable cytogenetic or molecular risk profile. The conditioning regimen consisted of hyperfractionated total body irradiation (1375cGy in eleven fractions) with partial lung shielding, thiotepa (10 mg/kg), cyclophosphamide (120 mg/kg), and rabbit antithymocyte globulin (2.5 mg/kg). The donors, all HLA-identical siblings, were given GCSF for mobilization and scheduled to undergo at least 2 leukapheresis procedures to ensure a graft with a high CD34+ cell content. All allografts were CD34 enriched and were targeted to contain > 5.0 x106 CD34+ cells/kg and < 1.0 x 105 CD3+ cells/kg. No pharmacological GVHD prophylaxis was given post-transplant.

The primary endpoint of the trial was met, in that the probability of disease-free survival (DFS) at six months post transplantation was 81.8% for all transplanted patients. Additionally, the data from the study showed that the performance of the CliniMACS® CD34 Reagent System was safe and consistent in this multicentric trial because of the reproducible, passive depletion of CD3+ cells (median 4.8 log10 depletion), the enrichment of CD34+ cells, through the sterility of the transplanted grafts and the absence of infusional toxicity and attributable adverse events and low rates of acute and chronic GVHD without the need for additional post-transplant GVHD prophylaxis.

A European safety study, ACS 950101, for the CliniMACS System was published in Bone Marrow Transplantation 25; 243-49, February 20001. 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 ofCD34+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 Table 2 (one subject died 5 days post-transplant and prior to engraftment and one subject 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 human murine antibodies (HAMA) production. A summary of the results of the European clinical trial is provided below.

Table 2: Time to Hematological Engraftment After Infusion of CliniMACS Selected CD34+ Cells		
Criteria for Engraftment	Time to Engraftment (Days)	
	Platelets ($\geq 20 \times 10^9$/L)	Neutrophils ($\geq 500/\mu L$)

Median (Kaplan –Meier)	11.6	9.1
Standard Deviation	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.

15.2 Anti-Thymocyte Globulin (equine) Sterile Solution (ATGAM®)

Other*:* Antithymocyte gammaglobulin, Antithymocyte globulin, ATGAM, Antithymocyte immunoglobulin, lymphocyte immune globulin and h-ATG.
Supply/availability: commercially available (Pharmacia & Upjohn Company).
Product description: Anti-thymocyte globulin (equine) sterile solution (ATGAM®) is available in 5 ml ampoules containing 50 mg of horse gamma globulin/mL (250 mg per ampoule).
Preparation*:* The calculated dose of anti-thymocyte globulin should be diluted in 0.9% sodium chloride injection to a concentration not to exceed 4 mg/mL.
Storage/stability*:* Anti-thymocyte globulin (equine) ampoules should be stored in a refrigerator at 2º to 8º C. Once diluted, anti-thymocyte globulin (equine) is physically and chemically stable for up to 24 hours at concentrations of up to 4 mg/mL in the recommended diluents. It is recommended that diluted anti-thymocyte globulin (equine) be stored in a refrigerator if it is prepared prior to the time of infusion.
Administration*:* Anti-thymocyte globulin (equine) should be administered into a high-flow central vein through an in-line filter with a pore size of 0.2 to 1 micron. The dose should be infused over no less than 4 hours. Infusion times may be extended to up to 24 hours for intolerance. Patients should be closely monitored for infusion / allergic reactions.
Compound*:* Principally monomeric IgG, prepared from plasma or serum of healthy horses hyperimmunized with human thymus lymphocytes.
Action: Immunosuppressive agent. Exact mechanism of immunosuppression of ATgam has not been fully elucidated but may involve elimination of antigen-reactive T-cells in peripheral blood and/or alteration of T-cell function.

15.3 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° 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.

15.4 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° Celcius (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.

15.6 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.

15.7 Mycophenolate Mofetil (MMF) (MMF, CellCept®)

Supply*:* Commercially available.
Product description*:* Oral dose formulations of mycophenolate mofetil include a 250 mg capsule, a 500 mg tablet, and an oral powder for suspension (200 mg/mL when reconstituted). The parenteral formulation of mycophenolate mofetil is available as a 500 mg vial containing a lyophilized powder that requires reconstitution.
Preparation*:* The parenteral formulation of mycophenolate mofetil requires both reconstitution and dilution with 5% dextrose injection (5% dextrose in water or D5W) in a two step process. Each 500 mg vial should first be reconstituted with 14 mL of 5% dextrose injection followed by gentle shaking of the vial to dissolve the drug. Once reconstituted, the prescribed dose should be further diluted with 5% dextrose injection to a final concentration of 6 mg/mL. Inspect the infusion solution for particulate matter or discoloration. Discard if particulate matter or discoloration is observed.
Storage and stability*:* Oral tablets, capsules and oral suspension should be stored at controlled room temperature 15-30ºC (59-86ºF). Once the oral suspension is reconstituted, the suspension may be stored at room temperature for up to 60 days. Refrigeration of the reconstituted oral suspension at 2-8ºC (36 to 46ºF) is also acceptable. Parenteral vials should also be stored at controlled room temperature 15-30ºC (59-86ºF). Parenteral doses following dilution with 5% dextrose injection are stable for 7 days under refrigeration (4ºC) or room temperature. NIH Clinical Center guidelines utilize a 48 hour expiration of prepared doses.
Route of administration: Oral or intravenous administration. For intravenous administration, each dose should be infused over a period of no less than 2 hours by either peripheral or central vein.

15.8 Prednisone
Supply: Commercially available.
Product description*:* Prednisone tablets are available in the following tablet sizes: 1 mg, 2.5 mg, 5 mg, 20 mg, and 50 mg. Prednisone is also available in an oral solution (5 mg per 5 ml).
Storage and stability*:* Store at controlled room temperature 15-30ºC (59-86ºF).
Route of administration*:* Oral.

15.9 Tacrolimus (FK506, Prograf®)

Supply*:* Tacrolimus will be obtained by the NIH Clinical Center Pharmacy Department from commercial sources and is available in capsules (0.5 mg, 1mg, and 5mg), and as a parenteral concentrate for injection (5 mg/ml, 1 ml ampules).
Preparation*:* For parenteral doses, tacrolimus injection concentrate (5 mg/ml) should be diluted to a final concentration of 0.004 to 0.02 mg/ml in dextrose 5% in water or sodium chloride 0.9%. Parenteral doses of tacrolimus will be prepared in non-PVC containers and infused with non-PVC administration sets/tubing
Storage and Stability*:* Capsules and ampules of parenteral concentrate bear expiration dates and are stored at room temperature. Tacrolimus conentrate for injection that has been diluted to a final concentration of 0.004 to 0.02 mg/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 sorption 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*:* Tacrolimus may be given intravenously over 24 hours or orally.

16. REFERENCES

1. Young, N.S. and A.J. Barrett, *The treatment of severe acquired aplastic anemia.* Blood, 1995. **85**(12): p. 3367-77.
2. Young, N.S. and J. Maciejewski, *The pathophysiology of acquired aplastic anemia.* N Engl J Med, 1997. **336**(19): p. 1365-72.
3. Zoumbos, N.C., et al., *Circulating activated suppressor T lymphocytes in aplastic anemia.* N Engl J Med, 1985. **312**(5): p. 257-65.
4. Platanias, L., et al., *Lymphocyte phenotype and lymphokines following anti-thymocyte globulin therapy in patients with aplastic anaemia.* Br J Haematol, 1987. **66**(4): p. 437-43.
5. Maciejewski, J.P., et al., *Bone marrow and peripheral blood lymphocyte phenotype in patients with bone marrow failure.* Exp Hematol, 1994. **22**(11): p. 1102-10.
6. Maciejewski, J.P., et al., *Increased expression of Fas antigen on bone marrow CD34+ cells of patients with aplastic anaemia.* Br J Haematol, 1995. **91**(1): p. 245-52.
7. Maciejewski, J., et al., *Fas antigen expression on CD34+ human marrow cells is induced by interferon gamma and tumor necrosis factor alpha and potentiates cytokine-mediated hematopoietic suppression in vitro.* Blood, 1995. **85**(11): p. 3183-90.
8. Risitano, A.M., et al., *Oligoclonal and polyclonal CD4 and CD8 lymphocytes in aplastic anemia and paroxysmal nocturnal hemoglobinuria measured by V beta CDR3 spectratyping and flow cytometry.* Blood, 2002. **100**(1): p. 178-83.
9. Bourdage, J.S. and D.M. Hamlin, *Comparative polyclonal antithymocyte globulin and antilymphocyte/antilymphoblast globulin anti-CD antigen analysis by flow cytometry.* Transplantation, 1995. **59**(8): p. 1194-200.
10. Raefsky, E.L., et al., *Biological and immunological characterization of ATG and ALG.* Blood, 1986. **68**(3): p. 712-9.
11. Sloand, E., et al., *Intracellular interferon-gamma in circulating and marrow T cells detected by flow cytometry and the response to immunosuppressive therapy in patients with aplastic anemia.* Blood, 2002. **100**(4): p. 1185-91.
12. Zeng, W., et al., *Characterization of T-cell repertoire of the bone marrow in immune-mediated aplastic anemia: evidence for the involvement of antigen-driven T-cell response in cyclosporine-dependent aplastic anemia.* Blood, 1999. **93**(9): p. 3008-16.
13. Gascon, P., et al., *Lymphokine abnormalities in aplastic anemia: implications for the mechanism of action of antithymocyte globulin.* Blood, 1985. **65**(2): p. 407-13.
14. Greco, B., et al., *Antithymocyte globulin reacts with many normal human cell types.* Blood, 1983. **62**(5): p. 1047-54.
15. Barrett, A.J., et al., *Crossreaction of antilymphocyte globulin with human granulocyte colony-forming cells.* J Clin Pathol, 1978. **31**(2): p. 129-35.
16. Di Bona, E., et al., *Rabbit antithymocyte globulin (r-ATG) plus cyclosporine and granulocyte colony stimulating factor is an effective treatment for aplastic anaemia patients unresponsive to a first course of intensive immunosuppressive therapy. Gruppo Italiano Trapianto di Midollo Osseo (GITMO).* Br J Haematol, 1999. **107**(2): p. 330-4.
17. Stein, R.S., et al., *Treatment of aplastic anemia with an investigational antilymphocyte serum prepared in rabbits.* Am J Med Sci, 1994. **308**(6): p. 338-43.
18. Bacigalupo, A., et al., *Antilymphocyte globulin, cyclosporin, and granulocyte colony-stimulating factor in patients with acquired severe aplastic anemia (SAA): a pilot study of the EBMT SAA Working Party.* Blood, 1995. **85**(5): p. 1348-53.
19. Rosenfeld, S.J., et al., *Intensive immunosuppression with antithymocyte globulin and cyclosporine as treatment for severe acquired aplastic anemia.* Blood, 1995. **85**(11): p. 3058-65.
20. Shevach, E.M., *The effects of cyclosporin A on the immune system.* Annu Rev Immunol, 1985. **3**: p. 397-423.

21. Orosz, C.G., et al., *Analysis of cloned T cell function. II. Differential blockade of various cloned T cell functions by cyclosporine.* Transplantation, 1983. **36**(6): p. 706-11.
22. Nussenblatt, R.B. and A.G. Palestine, *Cyclosporine: immunology, pharmacology and therapeutic uses.* Surv Ophthalmol, 1986. **31**(3): p. 159-69.
23. Brodsky, R.A., et al., *Durable treatment-free remission after high-dose cyclophosphamide therapy for previously untreated severe aplastic anemia.* Ann Intern Med, 2001. **135**(7): p. 477-83.
24. Tisdale, J.F., et al., *High-dose cyclophosphamide in severe aplastic anaemia: a randomised trial.* Lancet, 2000. **356**(9241): p. 1554-9.
25. Tisdale, J.F., et al., *Late complications following treatment for severe aplastic anemia (SAA) with high-dose cyclophosphamide (Cy): follow-up of a randomized trial.* Blood, 2002. **100**(13): p. 4668-70.
26. Storb, R., et al., *Changing trends in marrow transplantation for aplastic anemia.* Bone Marrow Transplant, 1992. **10 Suppl 1**: p. 45-52.
27. Horowitz, M.M., *Current status of allogeneic bone marrow transplantation in acquired aplastic anemia.* Semin Hematol, 2000. **37**(1): p. 30-42.
28. Tsai, T.W. and C.O. Freytes, *Allogenic bone marrow transplantation for leukemias and aplastic anemia.* Adv Intern Med, 1997. **42**: p. 423-51.
29. Margolis, D.A. and B.M. Cammita, *Hematopoietic stem cell transplantation for severe aplastic anemia.* Curr Opin Hematol, 1998. **5**(6): p. 441-4.
30. Storb, R., et al., *Cyclophosphamide combined with antithymocyte globulin in preparation for allogeneic marrow transplants in patients with aplastic anemia.* Blood, 1994. **84**(3): p. 941-9.
31. Deeg, H.J., et al., *Long-term outcome after marrow transplantation for severe aplastic anemia.* Blood, 1998. **91**(10): p. 3637-45.
32. Kahl, C., et al., *Cyclophosphamide and antithymocyte globulin as a conditioning regimen for allogeneic marrow transplantation in patients with aplastic anaemia: a long-term follow-up.* Br J Haematol, 2005. **130**(5): p. 747-51.
33. Gluckman, E., et al., *Bone marrow transplantation for severe aplastic anemia: influence of conditioning and graft-versus-host disease prophylaxis regimens on outcome.* Blood, 1992. **79**(1): p. 269-75.
34. Gluckman, E., et al., *Bone marrow transplantation in 107 patients with severe aplastic anemia using cyclophosphamide and thoraco-abdominal irradiation for conditioning: long-term follow-up.* Blood, 1991. **78**(9): p. 2451-5.
35. Deeg, H.J., et al., *Malignancies after marrow transplantation for aplastic anemia and fanconi anemia: a joint Seattle and Paris analysis of results in 700 patients.* Blood, 1996. **87**(1): p. 386-92.
36. Pierga, J.Y., et al., *Secondary solid malignant tumors occurring after bone marrow transplantation for severe aplastic anemia given thoraco-abdominal irradiation.* Radiother Oncol, 1994. **30**(1): p. 55-8.
37. Socie, G., et al., *Malignant tumors occurring after treatment of aplastic anemia. European Bone Marrow Transplantation-Severe Aplastic Anaemia Working Party.* N Engl J Med, 1993. **329**(16): p. 1152-7.
38. Socie, G., et al., *Increased incidence of solid malignant tumors after bone marrow transplantation for severe aplastic anemia.* Blood, 1991. **78**(2): p. 277-9.
39. Young, N.S., R.T. Calado, and P. Scheinberg, *Current concepts in the pathophysiology and treatment of aplastic anemia.* Blood, 2006. **108**(8): p. 2509-19.
40. Srinivasan, R., et al., *Overcoming graft rejection in heavily transfused and allo-immunised patients with bone marrow failure syndromes using fludarabine-based haematopoietic cell transplantation.* Br J Haematol, 2006. **133**(3): p. 305-14.
41. Kumar, R., et al., *Fludarabine, cyclophosphamide and horse antithymocyte globulin conditioning regimen for allogeneic peripheral blood stem cell transplantation performed in non-HEPA filter*

rooms for multiply transfused patients with severe aplastic anemia. Bone Marrow Transplant, 2006. **37**(8): p. 745-9.
42. Passweg, J.R., et al., *Bone marrow transplants from mismatched related and unrelated donors for severe aplastic anemia.* Bone Marrow Transplant, 2006. **37**(7): p. 641-9.
43. Deeg, H.J., et al., *Optimization of conditioning for marrow transplantation from unrelated donors for patients with aplastic anemia after failure of immunosuppressive therapy.* Blood, 2006. **108**(5): p. 1485-91.
44. Bacigalupo, A., et al., *Fludarabine, cyclophosphamide and anti-thymocyte globulin for alternative donor transplants in acquired severe aplastic anemia: a report from the EBMT-SAA Working Party.* Bone Marrow Transplant, 2005. **36**(11): p. 947-50.
45. Mao, P., et al., *Sustained and stable hematopoietic donor-recipient mixed chimerism after unrelated cord blood transplantation for adult patients with severe aplastic anemia.* Eur J Haematol, 2005. **75**(5): p. 430-5.
46. Cornetta, K., et al., *Umbilical cord blood transplantation in adults: results of the prospective Cord Blood Transplantation (COBLT).* Biol Blood Marrow Transplant, 2005. **11**(2): p. 149-60.
47. Laughlin, M.J., et al., *Hematopoietic engraftment and survival in adult recipients of umbilical-cord blood from unrelated donors.* N Engl J Med, 2001. **344**(24): p. 1815-22.
48. Rubinstein, P., et al., *Outcomes among 562 recipients of placental-blood transplants from unrelated donors.* N Engl J Med, 1998. **339**(22): p. 1565-77.
49. Laughlin, M.J., et al., *Outcomes after transplantation of cord blood or bone marrow from unrelated donors in adults with leukemia.* N Engl J Med, 2004. **351**(22): p. 2265-75.
50. Rocha, V., et al., *Transplants of umbilical-cord blood or bone marrow from unrelated donors in adults with acute leukemia.* N Engl J Med, 2004. **351**(22): p. 2276-85.
51. Takahashi, S., et al., *Single-institute comparative analysis of unrelated bone marrow transplantation and cord blood transplantation for adult patients with hematologic malignancies.* Blood, 2004. **104**(12): p. 3813-20.
52. Barker, J.N., et al., *Transplantation of 2 partially HLA-matched umbilical cord blood units to enhance engraftment in adults with hematologic malignancy.* Blood, 2005. **105**(3): p. 1343-7.
53. Nand, S. and J.E. Godwin, *Hypoplastic myelodysplastic syndrome.* Cancer, 1988. **62**(5): p. 958-64.
54. Jacobs, R.H., et al., *Prognostic implications of morphology and karyotype in primary myelodysplastic syndromes.* Blood, 1986. **67**(6): p. 1765-72.
55. Yunis, J.J., et al., *Refined chromosome study helps define prognostic subgroups in most patients with primary myelodysplastic syndrome and acute myelogenous leukaemia.* Br J Haematol, 1988. **68**(2): p. 189-94.
56. Morel, P., et al., *Cytogenetic analysis has strong independent prognostic value in de novo myelodysplastic syndromes and can be incorporated in a new scoring system: a report on 408 cases.* Leukemia, 1993. **7**(9): p. 1315-23.
57. Toyama, K., et al., *Clinical implications of chromosomal abnormalities in 401 patients with myelodysplastic syndromes: a multicentric study in Japan.* Leukemia, 1993. **7**(4): p. 499-508.
58. Greenberg, P., et al., *International scoring system for evaluating prognosis in myelodysplastic syndromes.* Blood, 1997. **89**(6): p. 2079-88.
59. Greenberg, P., *Myelodysplastic Syndrome*. 3rd ed. Hematology: Basic Principles and Practice, ed. R. Hoffman, Benz, EJ, Shattil, SJ. 2000, New York: Churchill Livingstone. 1106.
60. Heaney, M.L. and D.W. Golde, *Myelodysplasia.* N Engl J Med, 1999. **340**(21): p. 1649-60.
61. Mantovani, L., et al., *Treatment of anaemia in myelodysplastic syndromes with prolonged administration of recombinant human granulocyte colony-stimulating factor and erythropoietin.* Br J Haematol, 2000. **109**(2): p. 367-75.
62. Hellstrom-Lindberg, E., et al., *Treatment of anemia in myelodysplastic syndromes with granulocyte colony-stimulating factor plus erythropoietin: results from a randomized phase II study and long-term follow-up of 71 patients.* Blood, 1998. **92**(1): p. 68-75.

63. Negrin, R.S., et al., *Maintenance treatment of the anemia of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor and erythropoietin: evidence for in vivo synergy.* Blood, 1996. **87**(10): p. 4076-81.
64. Negrin, R.S., et al., *Treatment of the anemia of myelodysplastic syndromes using recombinant human granulocyte colony-stimulating factor in combination with erythropoietin.* Blood, 1993. **82**(3): p. 737-43.
65. Negrin, R.S., et al., *Treatment of myelodysplastic syndromes with recombinant human granulocyte colony-stimulating factor. A phase I-II trial.* Ann Intern Med, 1989. **110**(12): p. 976-84.
66. Negrin, R.S., et al., *Maintenance treatment of patients with myelodysplastic syndromes using recombinant human granulocyte colony-stimulating factor.* Blood, 1990. **76**(1): p. 36-43.
67. Molldrem, J.J., et al., *Antithymocyte globulin for treatment of the bone marrow failure associated with myelodysplastic syndromes.* Ann Intern Med, 2002. **137**(3): p. 156-63.
68. Sauntharajah, Y., et al., *HLA-DR15 (DR2) is overrepresented in myelodysplastic syndrome and aplastic anemia and predicts a response to immunosuppression in myelodysplastic syndrome.* Blood, 2002. **100**(5): p. 1570-4.
69. Sauntharajah, Y., et al., *A simple method to predict response to immunosuppressive therapy in patients with myelodysplastic syndrome.* Blood, 2003. **102**(8): p. 3025-7.
70. Steensma, D.P., et al., *Antithymocyte globulin has limited efficacy and substantial toxicity in unselected anemic patients with myelodysplastic syndrome.* Blood, 2003. **101**(6): p. 2156-8.
71. Jonasova, A., et al., *Cyclosporin A therapy in hypoplastic MDS patients and certain refractory anaemias without hypoplastic bone marrow.* Br J Haematol, 1998. **100**(2): p. 304-9.
72. Silverman, L.R., et al., *Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the cancer and leukemia group B.* J Clin Oncol, 2002. **20**(10): p. 2429-40.
73. Kornblith, A.B., et al., *Impact of azacytidine on the quality of life of patients with myelodysplastic syndrome treated in a randomized phase III trial: a Cancer and Leukemia Group B study.* J Clin Oncol, 2002. **20**(10): p. 2441-52.
74. Kantarjian, H.M., *Treatment of myelodysplastic syndrome: questions raised by the azacitidine experience.* J Clin Oncol, 2002. **20**(10): p. 2415-6.
75. List, A., et al., *Lenalidomide in the myelodysplastic syndrome with chromosome 5q deletion.* N Engl J Med, 2006. **355**(14): p. 1456-65.
76. Anderson, J.E., et al., *Allogeneic bone marrow transplantation for 93 patients with myelodysplastic syndrome.* Blood, 1993. **82**(2): p. 677-81.
77. De Witte, T., et al., *Allogeneic bone marrow transplantation for secondary leukaemia and myelodysplastic syndrome: a survey by the Leukaemia Working Party of the European Bone Marrow Transplantation Group (EBMTG).* Br J Haematol, 1990. **74**(2): p. 151-5.
78. Sutton, L., et al., *Factors influencing outcome in de novo myelodysplastic syndromes treated by allogeneic bone marrow transplantation: a long-term study of 71 patients Societe Francaise de Greffe de Moelle.* Blood, 1996. **88**(1): p. 358-65.
79. Nevill, T.J., et al., *Treatment of myelodysplastic syndrome with busulfan-cyclophosphamide conditioning followed by allogeneic BMT.* Bone Marrow Transplant, 1992. **10**(5): p. 445-50.
80. Anderson, J.E., et al., *Allogeneic marrow transplantation for refractory anemia: a comparison of two preparative regimens and analysis of prognostic factors.* Blood, 1996. **87**(1): p. 51-8.
81. Runde, V., et al., *Bone marrow transplantation from HLA-identical siblings as first-line treatment in patients with myelodysplastic syndromes: early transplantation is associated with improved outcome. Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation.* Bone Marrow Transplant, 1998. **21**(3): p. 255-61.
82. Anderson, J.E., F.R. Appelbaum, and R. Storb, *An update on allogeneic marrow transplantation for myelodysplastic syndrome.* Leuk Lymphoma, 1995. **17**(1-2): p. 95-9.

83. Kolb, H.J., et al., *Graft-versus-leukemia reactions in allogeneic chimeras.* Blood, 2004. **103**(3): p. 767-76.
84. Woods, W.G., et al., *A comparison of allogeneic bone marrow transplantation, autologous bone marrow transplantation, and aggressive chemotherapy in children with acute myeloid leukemia in remission.* Blood, 2001. **97**(1): p. 56-62.
85. Horowitz, M.M., et al., *Graft-versus-leukemia reactions after bone marrow transplantation.* Blood, 1990. **75**(3): p. 555-62.
86. Weiden, P.L., et al., *Antileukemic effect of graft-versus-host disease in human recipients of allogeneic-marrow grafts.* N Engl J Med, 1979. **300**(19): p. 1068-73.
87. Gorin, N.C., et al., *Retrospective evaluation of autologous bone marrow transplantation vs allogeneic bone marrow transplantation from an HLA identical related donor in acute myelocytic leukemia. A study of the European Cooperative Group for Blood and Marrow Transplantation (EBMT).* Bone Marrow Transplant, 1996. **18**(1): p. 111-7.
88. Zittoun, R.A., et al., *Autologous or allogeneic bone marrow transplantation compared with intensive chemotherapy in acute myelogenous leukemia. European Organization for Research and Treatment of Cancer (EORTC) and the Gruppo Italiano Malattie Ematologiche Maligne dell'Adulto (GIMEMA) Leukemia Cooperative Groups.* N Engl J Med, 1995. **332**(4): p. 217-23.
89. Feinstein, L., et al., *Nonmyeloablative hematopoietic cell transplantation. Replacing high-dose cytotoxic therapy by the graft-versus-tumor effect.* Ann N Y Acad Sci, 2001. **938**: p. 328-37; discussion 337-9.
90. Slavin, S., et al., *Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases.* Blood, 1998. **91**(3): p. 756-63.
91. Kroger, N., et al., *Allogeneic stem cell transplantation after a fludarabine/busulfan-based reduced-intensity conditioning in patients with myelodysplastic syndrome or secondary acute myeloid leukemia.* Ann Hematol, 2003. **82**(6): p. 336-42.
92. Martino, R., et al., *Evidence for a graft-versus-leukemia effect after allogeneic peripheral blood stem cell transplantation with reduced-intensity conditioning in acute myelogenous leukemia and myelodysplastic syndromes.* Blood, 2002. **100**(6): p. 2243-5.
93. T. De Witte, R.B.a.A.V.B.e.a., *Allogeneic stem cell transplantation with matched related and unrelated donors for patients with refractory anemia: T-cell depletion and reduced intensity regimens are associated with increased relapse risk.* Blood, 2003. **102**: p. 422a.
94. Arnold, R., et al., *Unrelated bone marrow transplantation in patients with myelodysplastic syndromes and secondary acute myeloid leukemia: an EBMT survey. European Blood and Marrow Transplantation Group.* Bone Marrow Transplant, 1998. **21**(12): p. 1213-6.
95. Powles, R.L., et al., *Mismatched family donors for bone-marrow transplantation as treatment for acute leukaemia.* Lancet, 1983. **1**(8325): p. 612-5.
96. Beatty, P.G., et al., *Marrow transplantation from related donors other than HLA-identical siblings.* N Engl J Med, 1985. **313**(13): p. 765-71.
97. Szydlo, R., et al., *Results of allogeneic bone marrow transplants for leukemia using donors other than HLA-identical siblings.* J Clin Oncol, 1997. **15**(5): p. 1767-77.
98. Aversa, F., et al., *Full haplotype-mismatched hematopoietic stem-cell transplantation: a phase II study in patients with acute leukemia at high risk of relapse.* J Clin Oncol, 2005. **23**(15): p. 3447-54.
99. Kim, H.J., et al., *Haplotype mismatched transplantation using high doses of peripheral blood CD34+ cells together with stratified conditioning regimens for high-risk adult acute myeloid leukemia patients: a pilot study in a single Korean institution.* Bone Marrow Transplant, 2005. **35**(10): p. 959-64.
100. Magro, E., et al., *Early hematopoietic recovery after single unit unrelated cord blood transplantation in adults supported by co-infusion of mobilized stem cells from a third party donor.* Haematologica, 2006. **91**(5): p. 640-8.

101. Rosenfeld, S., et al., *Antithymocyte globulin and cyclosporine for severe aplastic anemia: association between hematologic response and long-term outcome.* Jama, 2003. **289**(9): p. 1130-5.
102. Simon, R., *Optimal two-stage designs for phase II clinical trials.* Control Clin Trials, 1989. **10**(1): p. 1-10.

104 Kopp HG, Horger M, Faul C, Hartmann JT, Kanz L, Lang P, Vogel W, Granulocyte colony-stimulating factor induced pulmonary hemorrhage in a healthy stem cell donor. Granulocyte colony-stimulating factor induced pulmonary hemorrhage in a healthy stem cell donor. J Clin Oncol. 2007 Jul 20;25(21):3174-5.

104. Baker, F., & Kim, S. *The Basics of Item Response Theory* (2nd ed.). 2010; New York: Marcel Dekker.
Johnson J, Temple R. Food and Drug Administration requirement for approval of new anti-cancer drugs. *Cancer Treat Rep*. 1985;69:1155-1157.
105. PROMIS. (2011). Patient-Reported Outcomes Measurement Information System: Dynamic Tools to Measure Health Outcomes From the Patient Perspective
Retrieved January 31, 2011, from http://www.nihpromis.org/default.aspx
106. Riley, W. T., Rothrock, N., Bruce, B., Christodolou, C., Cook, K., Hahn, E. A., et al. Patient-reported outcomes measurement information system (PROMIS) domain names and definitions revisions: Further evaluation of content validity in IRT-derived item banks. *Quality of Life Research.* 2010 19(9), 1311-1321.
107. Institute of Medicine. Cancer Care for the Whole Patient: Meeting Psychosocial Health Needs. Washington, DC: IOM; 2008.
108. Lee S, Francis Cook E, Soiffer R, Anttin JH. Development and Validation of a Scale to Measure Symptoms of Chronic Graft-versus-Host Disease. *Biology of Blood and Marrow Transplantation.* 2002, 8:444-452.
109. Cella D: Manual of the Functional Assessment of Chronic Illness Therapy (FACIT Scales) (Version 4.1) 2004.
110. Cella D, Hernandez L, Bonomi AE, et al: Spanish language translation and initial validation of the functional assessment of cancer therapy quality-of-life instrument. *Med Care.* 1998; 36:1407-18.
111. Syrjala KL, Schroeder TC, Abrams JR, Atkins TZ, Sanders JE, Brown W, Heiman JR.Sexual function measurement and outcomes in cancer survivors and matched controls. *Journal of Sex Research.* 2000; 37(3) 213-225.
112. Geller NL, Follmann D, Leifer ES, Carter SL, Design of Early Trials in Stem Cell Transplantation: A Hybrid Frequentist-Bayesian Approach. In Advances in Clinical Trial Biostatistics (ed. Geller NL), 2004. New York: Marcel Dekker, Inc.
113. Lechowicz et al. Allogeneic hematopoietic cell transplantation for mycosis fungoides and Sezary syndrome. Bone Marrow Transplantation (2014) 49, 1360–1365.
114. Duarte RF et al. Allogeneic Hematopoietic Cell Transplantation for Patients With Mycosis Fungoides and Se´zary Syndrome: A Retrospective Analysis of the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. J Clin Oncol 2010.
115. Duarte RF et al. Long-Term Outcome of Allogeneic Hematopoietic Cell Transplantation for Patients With Mycosis Fungoides and Se´zary Syndrome: A European Society for Blood and Marrow Transplantation Lymphoma Working Party Extended Analysis. J Clin Oncol 2014.

APPENDIX A: IRB APPROVED NHLBI LABORATORY RESEARCH STUDIES
v. 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
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 inoculation 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 Grading of GVHD

GVHD is staged based on degree of severity of skin, liver and gastrointestinal (GI) tract involvement ranging from mild to severe (table 1). Acute GVHD is then graded for prognostic and treatment purposes based on a four-grade scale, with grade I considered mild, grade II as moderate, grade III as severe, and grade IV being a life-threatening (Table 2). The likelihood of severe and life-threatening acute GVHD increases with the degree of mismatch between the major histocompatibility complex (MHC) antigens of the donor and those of the recipient as well as factors such as donor and recipient age, cytomegalovirus (CMV) donor and recipient status, underlying disease, conditioning regimen and type of GVHD prophylaxis. For the purposes of secondary endpoint assessment the following grading schemas will be used:

Table 1: CLINICAL GRADING. Organ systems are staged for severity as follows:

SKIN				
Stage	1	2	3	4
Area affected	<25	25-50	>50%	exfoliation
GASTROINTESTINAL TRACT				
Stage	1	2	3	4
Diarrhea (l/day)	<0.5	<1.0	<1.5	ileus, blood loss
LIVER				
Stage	1	2	3	4
Bilirubin mg/dl	3	3-6	6-15	>15

Table 2: OVERALL GRADING is made from the individual organ stages as follows:

Skin	Gut /Liver	GRADE	
0	0	0	
1	0	I	not clinically significant
2	0	II	clinically significant
0-2	1-2		
3	0-2	III	
0-3	3		
Any	4	IV	