AN EXPANDED ACCESS STUDY USING THE CLINIMACS® SYSTEM TO OFFER THERAPEUTIC MANIPULATED GRAFTS THAT ARE CD34 CELL ENRICHED AND T CELL DEPLETED FOR ALLOGENEIC STEM CELL RECIPIENTS WITH MISMATCHED OR MATCHED RELATED OR UNRELATED DONORS OR BORDERLINE ORGAN FUNCTION

**Division of Pediatrics: Stem Cell Transplantation Section**

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|---|---|---|
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**Protocol Synopsys:**

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| **Funding Source** | None. |
| **Protocol Title** | An Expanded Access Study Using the CliniMACS® System to Offer Therapeutic Manipulated Grafts that are CD34+ Cell Enriched and T Cell Depleted for Allogeneic Stem Cell recipients with Matched or Mismatched Related Donors or with Borderline Organ Function |
| **Clinical Phase** | Phase II |
| **Background and Rationale** | **Hypothesis:** Transplantation of stem cells that have been CD34+ selected and T cell-depleted with the CliniMACS® device will prevent severe (grade III/IV) acute GvHD without the use of prophylactic post-transplant immunosuppression. The incidence of grade III/IV acute GVHD is predicted to be <10% after cell manipulation.<br><br>**Background:** The CliniMACS® System (Device, Reagents, Tubing Sets, Instruments and PBS/EDTA Buffer) is manufactured and controlled under an ISO 13485 and ISO 9001 certified quality system. In Europe, the CliniMACS System and Reagents are available as CE-marked medical devices for use in humans. Inthe USA, CliniMACS® System is available for use only under an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE). Its safety has been tested in multiple eIND and eIDE FDA submissions. As of 2011, eighty four IDE protocols utilize the CliniMACS® CD34 Reagent to select CD34+ cells from heterogeneous hematologic cell populations. In 2011, Miltenyi Biotech HDE submitted CliniMACS® CD34 Reagent System as a Humanitarian Use Device (HUD) in its Registration Pathway in the U.S. Clinical data from BMT CTN 0303 Clinical – a phase II multi-center clinical trial sponsored by the BMT CTN (BB-IDE 11965) which enrolled 47 Acute Myeloid Leukemia (AML) patients after myeloablative therapy for HLA-matched allogeneic stem cell transplant recipients, without additional GvHD prophylaxis from October 2005 to December 2008 -- supports “safety” and “probable benefit” arguments of the CliniMACS® System. In January, 2014, the CliniMACS CD34 Reagent System received FDA approval as a Humanitarian Use Device for processing hematopoietic progenitor cells collected by apheresis from an allogeneic, HLA-identical, sibling donor to obtain a CD34+ cell-enriched population for hematopoietic reconstitution following a myeloablative preparative regimen without the need for additional GvHD prophylaxis in acute myeloid leukemia. (44)<br><br>**Rationale:** Hematopoietic stem cell transplantation (HSCT) is now recognized as an effective form of therapy for an increasing number of malignant and non- malignant disorders. Therefore, extending the donor pool to alternative source of hematopoietic stem cells (HSCs) that is capable of reconstituting hematopoiesis after intensive myeloablative therapy is an important goal in this field. The preferred donor for an allogeneic HSCT is an HLA identical sibling. However, less than 25% of patients requiring a transplant will have such a donor. The purpose of this protocol is to provide access to the CliniMACS® System to patients who only have donors with a significant HLA-mismatched compatibility (alternative donors) or have a matched related donor but cannot receive post immunosuppressive therapy due to organ function. By manipulating the product graft through this platform, we can offer a CD34 enriched and T-cell depleted graft that addresses the main morbidity and mortality issue for this type of hematopoietic stem cell transplant (HSCT): overwhelming (grade III/IV) graft-versus-host disease (GvHD). The cell manipulation provides cellular therapeutic GvHD prophylaxis, therefore |

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| | eliminating the need of post-HSCT immunosuppressant therapies making this process also a viable alternative to deliver a life-saving HSCT for patients who do not have a matched related donor and for those with borderline organ function -- who might not endure the often-toxic side-effects of traditional post-HSCT immunosuppressive therapy. |
| **Objectives** | This is a prospective study to provide access to an investigational device and reagents used for progenitor cell sorting of a stem cell transplant donor graft to determine the ability of CD34+ cell selection and T Cell depletion (using the CliniMACS® device as the sole GvHD prophylaxis) to prevent severe (grade III-IV) acute GvHD in recipients of mismatched related or unrelated donor hematopoietic stem cell transplants or with borderline organ function -- who might not endure the often toxic side-effects of traditional post-HSCT immunosuppressive therapy. |
| **Primary Endpoint(s)** | The primary endpoint of the study is the incidence of severe (grade III/IV) acute GvHD occurring by Day +100 after transplant. |
| **Secondary Endpoint(s)** | Outcomes measures for the secondary endpoints will include:<br>Engraftment: The incidence of primary graft failure and graft rejection will be determined.<br>Chimerism at Day 100: The percentage of donor cells will be reported for all evaluable (without disease progression) patients.<br>Immune recovery: The time to CD4 count >100 and > 200 and PHA ≥ 10% and 30% will be calculated.<br>Severe toxicities: The incidence of severe toxicities will be determined. This will include grade 3/4 stem cell product infusion-related toxicity.<br>Post-transplant infections (bacterial, viral and fungal): Post-transplant infections will be described by incidence and type.<br>Post-transplant lymphoproliferative disease (PTLD): The incidence of PTLD will be calculated.<br>Transplant-related mortality (TRM): The incidence of TRM will be calculated at Day 100 after transplant and at one year.<br>Disease-free survival.<br>Overall survival: Overall survival will be estimated using the Kaplan-Meier method.<br>Device (CliniMACS®) performance parameters: The parameters will be summarized for all products that are processed and median and ranges will be determined. |
| **Study Design** | Participants will be enrolled with alternative (mismatched/haplocompatible) related/unrelated donors or matched related/unrelated donors for an initial HSCT. |
| **Study Centers** | This is a Stanford University, Principle Investigator (PI) initiated protocol conducted at LPCH and Stanford Hospital and Clinics. |
| **Duration of Study per Subject** | Individual participants will be followed for outcomes related to the experimental device including acute and chronic GvHD and engraftment for a minimum of one year. Subsequent follow-up care will follow the standard for all transplant patients. |
| **Subject Population** | Pediatric and adult patients up to 35 years old, of any gender, and ethnic background, with malignant and/or non-malignant diagnosis that are treatable though allogeneic stem cell transplantation. |

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<tr>
<td>Eligibility criteria</td>
<td>Inclusion Criteria:<br>• Participants greater than one month of age.<br>• Participant has a disorder that can be treated with a hematopoietic stem cell transplant<br>Participant's is eligible for an allogenic HSCT as per current institutional SOP and FACT standards<br>• Participant must have a related or unrelated matched or mismatched-related donor who is:<br>o Able to receive granulocyte colony-stimulating factor (G-CSF) and undergo apheresis either through placement of catheters in antecubital veins or a temporary central venous catheter OR agrees on a bone marrow harvest;<br>o Healthy as per donor selection screening (following current SOP based on standards of foundation for accreditation of cellular therapy and stem cell transplantation – FACT);<br>o Willing to participate and sign consent.<br>• Participant or Legal Authorized Representative is able to sign informed consent (and signed assent, if applicable) for transplant.<br>Exclusion Criteria:<br>• Participant does not qualify for an allogeneic transplant due to medical screening, underlying disease, or lack of alternative donors.<br>• Any condition that compromises compliance with the procedures of this protocol, as judged by the principal investigator.</td>
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<td>Discontinuation Criteria (Safety Considerations)</td>
<td>Principal Investigator will evaluate and report all grade 3 or 4 adverse reactions that are unexpected and possibly related to the investigation in process and are not expected TRM as per protocol definition. If it becomes clear that the study treatment is less effective than other available treatments or the toxicity is unacceptable we will follow the stopping rules.<br><br>Stopping rule for primary graft failure: The first 30 enrolled patients (related and unrelated) will be evaluated for the stopping rule for primary graft failure. The trial is stopped if there are ≥ b graft failures out of k resolved patients. Only points where stopping is possible are listed.<br><br>
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<br>The stopping rule for graft failure yields the probability of stopping the trial of 0.05 if the rate of graft failure is 0.1. The probability of stopping the trial is 0.41 if the graft failure rate is 0.2, 0.82 if the graft failure rate is 0.3, and 0.96 if the graft failure rate is 0.4. These probabilities were calculated based on the binomial distribution.<br><br>If the study reaches a stopping boundary, the study will be suspended. Proper use of the stopping rule table will be ensured by the Study Investigator.<br><br>Stopping rule for Grade ≥ IV toxicity: If any patient develops grade ≥ IV acute GvHD, grade ≥ IV infection, or any unexpected grade ≥ IV toxicity by 4 weeks post-transplant, the protocol will be halted and the processing re-evaluated before</td>
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| | proceeding.<br><br>Conditions for individual patient termination:<br>Disease progression<br>Need for exclusionary concurrent treatment (per PI discretion)<br>Withdrawal of informed consent<br>Protocol non-compliance<br><br>The PI, through the reports from the Stem Cell Laboratory, will also monitor system performance based on:<br>Cellular composition of final product<br>Yield (% recovery) of CD34+ cells from the starting product<br>CD3+ cell reduction<br>Sterility<br>Purity |
| **Sample Size** | The study will enroll 30 transplant recipients at Stanford. |
| **Statistical Methodology** | Statistical analysis results will be reported using summary tables, figures, and data listings. Continuous variables will be summarized using the mean, standard deviation, median, minimum, and maximum. Categorical variables will be summarized by numbers and percentages of subjects in corresponding categories. All raw data obtained from the case report form (CRF) and any derived data will be summarized and included in data listings. Missing data will not be imputed. |

**List of Abbreviations and Definition of Terms**

| **List of Abbreviations** | |
|---|---|
| AE | Adverse event |
| AdV | Adenovirus |
| aGvHD | Acute Graft-versus-Host Disease |
| ALL | Acute Lymphoblastic Leukemia |
| Allo | Allogeneic |
| AML | Acute Myelogenous Leukemia |
| ANC | Absolute Neutrophil Count |
| BM | Bone marrow |
| BM bx. | Bone marrow biopsy |
| BMI | Body mass index |
| BMT | Bone Marrow Transplant |
| BP | Blood pressure |
| BSA | Body surface area |
| $^{o}C$ | Degrees Celsius |
| CB | Cord blood |
| CBC | Complete blood count |
| CD | Cluster of Differentiation |
| CFR | Code of Federal Regulations |
| cGvHD | Chronic Graft-versus-Host Disease |
| CIBMTR | Center for International Blood and Marrow Transplant Research |
| CML | Chronic Myelogenous Leukemia |
| CMV | Cytomegalovirus |
| CNS | Central nervous system |
| Co-PI(s) | Co-Investigator(s) |
| CRF | Case report/record form |
| CR | Complete response |
| CTCAE | Common Terminology Criteria for Adverse Events |
| CTN | Clinical Trials Network |
| DFS | Disease Free Survival |
| DLI | Donor Leukocyte Infusion |
| DLT | Dose Limiting Toxicity |
| DSMB | Data Safety Monitoring Board |
| DSMC | Data and Safety Monitoring Committee |
| EBV | Epstein Barr Virus |
| EC | Ethics Committee |
| ECG | Electrocardiogram |
| Echo/ECCO | Echocardiogram |

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| EFS | Event-free survival |
| EIDE | Emergency investigational device exemption |
| EIND | Emergency Investigational new drug |
| F | Fahrenheit |
| FA | Fanconi Anemia |
| FACT | Foundation for the Accreditation of Cellular Therapy |
| FDA | Food and Drug Administration |
| GCP | Good clinical practice |
| G-CSF | Human granulocyte-colony stimulating factor |
| GI | Gastrointestinal |
| GFR | Glomerular filtration rate |
| GvHD | Graft-versus-Host Disease |
| HBV | Hepatitis B Virus |
| HCG | Human chorionic gonadotropin |
| HCV | Hepatitis C Virus |
| Hgb | Hemoglobin |
| HIV | Human Immunodeficiency Virus |
| HHV | Human Herpes Virus |
| HLA | Human leukocyte antigen |
| HPF | High-power field |
| HR | Heart rate |
| HSCs | Hematopoietic stem cells |
| HSCT | Hematopoietic Stem Cell Transplant |
| HSV | Herpes Simplex Virus |
| HTN | Hypertensions |
| HUD | Humanitarian Use Device |
| IB | Investigator Brochure |
| IDE | Investigational Device Exemption |
| IND | Investigational New Drug |
| IRB | Institutional Review Board |
| IV | Intravenous |
| LAR | Legal authorized representative |
| LLN | Lower limit of normal |
| $LN_2$ | Liquid Nitrogen |
| mAb | Monoconal antibody |
| MDS | Myelodysplastic Syndrome |
| MRD | Matched related donor |
| NCI | National Cancer Institute |
| NCI CTCAE | National Cancer Institute common terminology criteria for adverse events |
| NHL | Non-Hodgkin’s Lymphoma |

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|---|---|
| NIH | National Institutes of Health |
| NK | Natural killer |
| NMDP | National Marrow Donor Program |
| OS | Overall survival |
| PBSC | Peripheral blood stem cells |
| PCR | Polymerase chain reaction |
| PD | Progressive diseased |
| Peds | Pediatric patients |
| PFS | Progression free survival |
| PI | Principal investigator |
| PLT | Platelet |
| PMRD | Partially matched donor |
| PPA | Per protocol analysis |
| PR | Partial response |
| PTLD | Post-transplant lymphoproliferative disorder |
| RR | Response rate |
| SAE | Serious adverse event |
| SCCI | Stanford Center for Clinical Informatics |
| SD | Stable disease |
| SCT | Stem cell transplant |
| SCTT | Stanford Blood and Marrow Cellular Therapeutics & Transplantation Laboratory |
| SOP | Standard Operating Procedure |
| SRC | Scientific Review Committee |
| T. bili | Total bilirubin |
| TBI | Total body irradiation |
| TRM | Transplant-related mortality |
| TTP | Time to progression |
| UCB | Umbilical Cord Blood |
| ULN | Upper limit of normal |
| UNK | Unknown |
| URD | Unrelated donor |
| VZV | Varicella Zoster Virus |
| WBC | White blood cell |
| WHO | World Health Organization |

## 1.0 OBJECTIVES

A major issue in alternative donor (mismatched related and unrelated donor) transplantation is the development of graft-versus-host disease (GvHD). For those who cannot receive post-transplant immunosuppressive therapy, any form of graft versus host disease, even with a matched related or unrelated donor can also be a complication. Several clinical trials have shown that the use of T cell-depleted peripheral blood stem cells (PBSC) reduces GvHD in hematopoietic stem cell transplants (HSCT). By using a purified stem cell graft, there is a reduced risk of post-transplant graft versus host disease in patients who have a mismatched donor or cannot receive post-transplant immunosuppression.

This is a prospective study to provide access to an investigational device and reagents used for progenitor cell sorting. The hypothesis is that the CliniMACS® System will allow for a manipulated graft – with augmented CD34+ cells and T cell-depleted numbers --- in a ratio that will prevent severe (grade III/IV) acute GvHD (as defined on appendix C) without the use of prophylactic post-transplant immunosuppression. The incidence of grade III/IV acute GVHD is predicted to be lesser than 10% based on similar ongoing trials (17).

The objective is to manipulate (alter the ratio) specific cells in the donor graft to determine the ability of augmented CD34+ cells and T cell-depleted products to prevent severe (grade III/IV) acute GvHD in recipients of mismatched related donor hematopoietic stem cell transplants as well as to candidates of allogeneic (allo) transplant with borderline organ function -- who might not endure the often toxic side- effects of traditional post-HSCT immunosuppressive therapy.

The patients will receive conditioning therapy based on their diagnosis and per instructional guidelines for treating the disease. The transplant recipients will be followed for one year post- transplant for the development of GvHD, engraftment, post-transplant infections, disease relapse, and overall survival.

### 1.1 Primary Objective

To determine the ability of CD34+ cell selection using the CliniMACS® device as the sole GvHD prophylaxis to prevent severe (grade III-IV) acute GVHD in recipients of alternative donors hematopoietic stem cell transplants and/or potential matched-related allogeneic SCT recipients with borderline organ function (who might not tolerate traditional post-HSCT GvHD prophylaxis without increased morbidity).

### 1.2 Secondary Objectives

Assess the ability of this approach to serve as a platform for strategies to accelerate post- transplant immunological recovery.

Evaluate chimerism at day +100 (from the date of transplant graft infusion), and the rate of engraftment in recipients of CD34+ cell selected, T cell-depleted transplants from mismatched related donors.

Evaluate the rates of post-transplant occurrences, including:

a) Graft rejection and graft failure
b) Immune recovery
c) Transplant-related severe toxicities
d) Post-transplant infections
e) CMV infection and disease
f) EBV-related post-transplant lymphoproliferative disorder (PTLD)
g) Relapse of malignant-diagnosis
h) Transplant-related mortality
i) Disease-free survival (DFS) and overall survival (OS)

Monitor device performance:

a) Cellular composition of final product
b) Yield of CD34+ cells
c) CD3+ cell depletion
d) Sterility
e) Purity

## 2. BACKGROUND

### 2.1 Previous Studies

Hematopoietic stem cell transplantation (HSCT) is now recognized as an effective form of therapy for an increasing number of malignant and non-malignant disorders. Therefore, extending the donor pool to alternative sources of hematopoietic stem cells (HSCs) that are capable of reconstituting hematopoiesis after intensive myeloablative therapy is an important goal in this field. The preferred donor for an allogeneic HSCT is an HLA identical sibling. However, less than 25% of patients requiring a transplant will have such a donor. Alternative stem cell sources include volunteer matched unrelated donors (URD) and unrelated donor cord blood (CB).(1) For patients lacking either a matched sibling donor or a suitable unrelated donor in a timely fashion, mismatched related donors could be a potential option.

To overcome the lack of suitable donors, transplant centers around the world started using mismatched (haplocompatible or sharing only one of two haplotypes) related donors. In the past, the major challenges have been engraftment, graft-versus-host disease (GvHD), and an increased incidence of infection and disease relapse.

Outcomes have improved markedly in recent years with the availability of cell selection devices that allow the administration of a large number of stem cells with a low dose of T cells and the development of more intensely immunosuppressive conditioning regimens. In an effort to reduce the high risk of fatal GvHD associated with these mismatched donors, the stem cells need to be processed to significantly reduce the number of donor T cells present in the graft. CD34 is a receptor that is expressed on early hematopoietic stem cells (HSC). Monoclonal antibodies are available that efficiently bind to the CD34 antigen. Studies have shown that positive selection of CD34+ marrow or blood cells results in a significant (> 4 log) depletion of T cells from the preparation (8-10). This approach is less time-consuming and may be more efficient than earlier approaches because it specifically targets the HSC. A cell separation system for clinical use is available for evaluation in the US after extensive use in Europe (11,12). The Miltenyi Biotec Inc. CliniMACS® CD34+ Reagent System has the advantages of the best T cell depletion efficiency achievable and a very high efficiency of CD34+ cell recovery so that fewer apheresis are necessary. The CliniMACS® device uses a sterile, closed magnetic sorting system to isolate CD34+ stem cells.

The bone marrow or peripheral blood stem cell product obtained from the donor is incubated with a murine anti-CD34 monoclonal antibody conjugated to small super- paramagnetic beads composed of iron dextran (commercially available to treat iron deficiency). The murine antibody has been used in clinical trials in humans. The dose administered to the transplant after processing is 100 times lower than therapeutic levels. Following incubation, unbound reagent is removed by washing the cell product and the product loaded onto the CliniMACS®. The cells are passed through a strong magnetic field applied to the CliniMACS® column and the CD34+ cells retained on the column while other cells (T, B, and NK cells, monocytes, and neutrophils) pass through into a waste bag. The magnet is withdrawn and the CD34+ cells eluted from the demagnetized column into a blood transfer pack for final formulation prior to release for infusion.

It has been well established that allogeneic peripheral blood stem cells (PBSC) recruited into the blood by administering G-CSF can successfully and durably engraft in both matched (13,14) or mismatched (15,16) transplant settings. PBSC recruited with cytokines generally engraft earlier and contain a larger number of HSC compared to marrow HSC (14,15).

Much of the work that has been done with this approach has been reported by a group in Perugia, Italy. They used the CliniMACS® device to lower the T cell content of the donor PBSC to a median of $1 \times 10^4$/kg (range 0.04 – $3 \times 10^4$/kg). Ninety-one percent of patients had primary engraftment. Six of seven patients who did not have primary engraftment were successfully engrafted after a second transplant, making the overall engraftment rate 99%. Grade III-IV acute GvHD occurred in 2% of patients. The transplant-related mortality was 37%, with the majority of deaths due to infection (bacterial, viral, and fungal). Relapse occurred in 16% of 66 patients who were in remission at the time of transplant. The two-year EFS for patients in remission was 48% for AML and 46% for ALL (17).

The results of haplocompatible transplantation in children have been encouraging. Handgretinger reported a 46%

two years EFS in patients with ALL in CR1-3 (18) Ortin et al. reported 75% EFS of patients with ALL in CR2/3 with median follow-up of 18 months (range 6-29) (19). The international experience was reviewed at a conference in Naples in 2004. Lang updated the Tubingen, Germany experience when he reported a 44% 2 years EFS for 21 children with ALL in CR1-3 (20)

Advantages of haplocompatible transplantation are rapid engraftment and the very low incidence of severe acute GVHD. Ortin et al. reported a median time to ANC > 500 and to platelet count > 50,000 of 12 and 20 days, respectively, with the incidence of grade III/IV acute GvHD being 5% (19). Lang reported a 1% incidence of grade III/IV acute GvHD (21).

In a retrospective review of the experience of 3 investigators in the U.S. (Cowan, Gilman, Sleight) with alternative (haplocompatible) donor transplant using CD34+ cell selection for T cell depletion, 13/18 (72%) were surviving with follow-up of 7 months – 7 years (median 31 months)(22). The median patient age was 8 years (range 1-20). Patient with malignancy (n=13) included: AML - CR1 (primaryinduction failure, failed cord blood transplant) [1], CR2 [3]; MDS - RA/RARS [2], RAEB [2]AM (and Fanconi anemia, FA) [1]; CML - CP2 [1]; ALL - CR3 [2]; NHL - CR2 [1]. Patients with non-malignant (n=5) disease included severe aplastic anemia (1 with prior bone marrow transplant three years earlier) [4] and Wiskott-Aldrich syndrome [1].

Fourteen donors were a 3/6 HLA match and 5 were a 4/6 match (one patient had two transplants using different donors). A CD34-positive selection device – Miltenyi CliniMACS® (15), Isolex (3) – was used to select stem cells and deplete T lymphocytes. Patients received a median of 18 x $10^6$ CD34+ (stem) cells/kg (range 6-28) and 3 x $10^4$ CD3+ (T) cells/kg (range 0.3-11).

Sustained primary engraftment occurred in 15/18 (83%) patients. Primary graft failure occurred in one patient. Two patients had immunological rejection following HHV-6 reactivation. They both engrafted after a second transplant; therefore the overall engraftment rate was 94%. The median time to an ANC >0.5 x $10^9$/L was 12 days (range 9-21). Platelet recovery occurred in 16/18 at a median of 17 days (range 9-22). Grade II acute GvHD was seen in 4/17 patients (24%). Grade III-IV acute GvHD was seen in 1 pt. (6%) with overlap syndrome (acute + chronic GvHD) associated with HHV-6 reactivation. Nine patients received DLI and/or stem cell boosts (boosts for graft rejection); 4 had grade II GvHD (3/4 had a history of acute GvHD) and none had grade III-IV GvHD. After DLI and/or stem cell boost, two patients developed extensive chronic GvHD and one developed overlap syndrome. The day 100 mortality and one year transplant-related mortality were 11% and 19%, respectively. Four patients (of 13 at risk, 31%) have relapsed; 1 patient with cytogenetic relapse is in CR > 1 year later. The 2 year predicted survival is 64% (60% for 13 patients with malignant disease and 75% for 5 patients with non- malignant disease).

Infections were common. All patients were at risk for CMV reactivation. Seven patients (39%) reactivated CMV. All cases were responsive to anti-viral therapy and/or DLI. No CMV disease was seen. Seven patients had adenovirus reactivation and six had HHV-6 reactivation. EBV reactivation occurred in 5/18 (28%) patients, three of whom manifested signs of post-transplant lymphoproliferative disorder.
Patients received a median of 3 x $10^4$ CD3+ cells/kg at the time of transplant. Some patients received additional donor T cells (DLI) for viral reactivation. At three months post-transplant, only 4 of the 15 evaluable patients had a CD4 count > 100. By nine months post-transplant, 10 of the 13 evaluable patients had a CD4 count > 200.

There is an ongoing study at the University of California, San Francisco Medical Center of a prospective trial of CD34+ selected (with the CliniMACS® device) PBSC from mismatched related donors (protocol CC# 01151). Patients received a conditioning regimen including TBI 1200 cGy, thiotepa, fludarabine, and rabbit-ATG (3.5 mg/kg). Patients received a fixed T cell dose of 3 x $10^4$/kg at the time of transplant. Seventeen evaluable patients have been reported with the following diagnoses: ALL (3), AML (6), biphenotypic leukemia (1), CML (1), MDS (1), aplastic anemia (2), congenital amegakaryocytic thrombocytopenia (1) combined immunodeficiency (1), and hemophagocytic lymphohistiocytosis (1).

Twelve of the 17 (65%) were alive and well as of 2010, with follow-up ranging from 3 months to 6.5 years (median follow-up 2.5 years). Survivors include 7/12 (58%) with malignant disease and 5/5 (100%) with non-malignant disease. One patient died at 7 months after transplant due to parainfluenza and Paecilomyces infections and

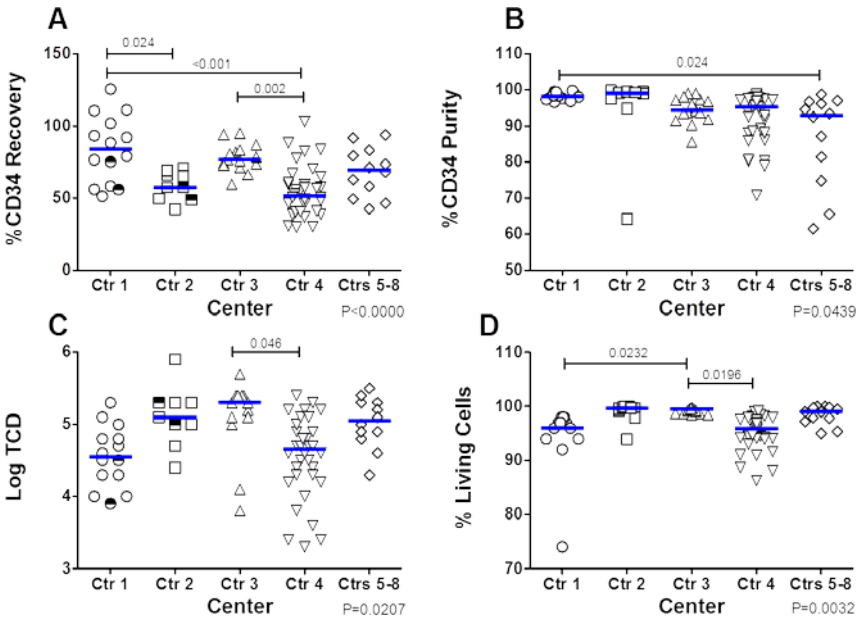
another died at 23 months after transplant due to disseminated Mucor infection, a fungus from the class of Zygomycetes. There was no severe (grade III/IV) acute GvHD. Only 3/17 (18%) of patients achieved > 100 CD4+ T cells/uL by 100 days after transplant and 3/4 of these patients either had received donor T cell infusion for serious viral infections prior to 100 days or had GvHD (in which case the T cells probably represented those causing the GvHD and not providing protection against infection).

A variety of genetic diseases of lymphohematopoiesis have been effectively treated and either cured or ameliorated by allogeneic HSCT from an HLA-matched histocompatible healthy sibling [45, 46]. The conditioning regimen of myeloablative high dose Busulfan along with immunoablative high-dose cyclophosphamide (BU- CY) ± anti-thymocyte globulin (ATG) was developed in the 1980's after important clinical studies were conducted to better understand the prerequisites for replacement of hematopoietic stem cells (HSC) carrying deleterious mutations with those derived from matched healthy donors [46]. These studies established that, in the setting of histocompatible transplant, ablation of host hematopoietic stem cells with high-dose BU (16 mg/kg, 600 mg/m2, or 640 mg/m2) and of host immunity with CY ± ATG allowed durable engraftment of donor HSCs.

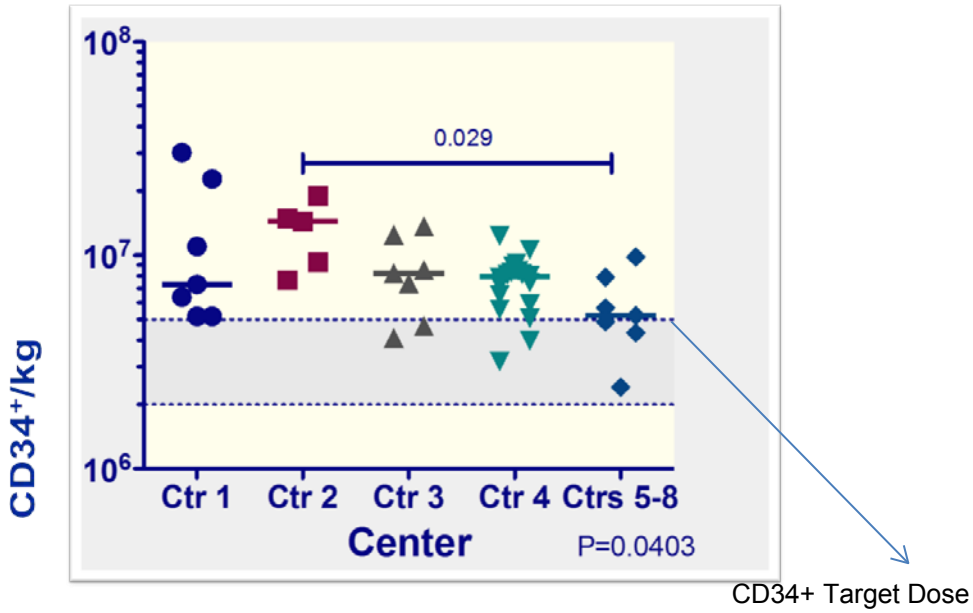
Since most patients lack a histocompatible sibling donor, a major challenge in the treatment of the genetic lymphohematological disorders is the development of HSCT regimens that result in high levels of engraftment while avoiding regimen-related toxicity and GVHD. Higher risks of graft rejection and GVHD with the use of alternative donors, including unrelated donors (URD), mismatched related donors (mMRD), umbilical cord blood units, and haplo-identical donors, makes these transplants high risk and are associated with high rates of TRM as well. Consequently, the results of HSCT for patients with genetic disorders, using alternative donors has been both limited and suboptimal [47-54].

Given the especially increased risk of rejection with haploidentical transplants in patients with non-malignant disorders [52,54] , we are proposing the use of additional immunosuppression to the standard back-bone of Busulfan/cyclophosphamide/anti- thymocyte globulin, by adding Fludarabine.

Miltenyi supported a Phase II multi-center clinical trial sponsored by the BMT CTN (BB-IDE 11965) which enrolled 47 AML patients from October 2005-December 2008 Evaluated the use of the CliniMACS® CD34 Reagent System for selecting CD34+ cells from HLA-matched related donors for allogeneic stem cell transplantation after myeloablative therapy in patients with Acute Myeloid Leukemia (AML) in 1st or 2nd CR, without additional GvHD prophylaxis




Post processing outcomes in all centers were able to process grafts that met the study criteria.



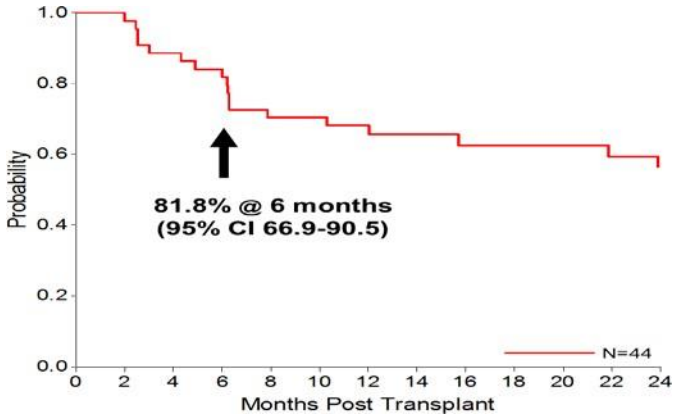

- All patients received the minimum CD34+ dose (> 2.0 x $10^6$ cells/kg)
- 84.1% of patients received > 5 x $10^6$ CD34+ cells/kg

**Final Cellular Product Summary**

| Parameter | Result |
|---|---|
| Median $CD34^+$ dose | 7.92 x $10^6$/kg |
| Range | 2.4 - 30.3 x $10^6$/kg |
| | |
| Median $CD3^+$ dose | 0.7 x $10^4$/kg |
| Range | 0.1 – 8.3 x $10^4$/kg |
| | |
| Median $Log_{10}$ TCD | 4.9 logs |
| Range | 3.2 – 5.9 logs |

- All Gram stains/14 day cultures were negative
- All endotoxin < 5.0 EU/kg
- No significant infusion related toxicities observed

Primary Endpoint 6 Month Disease-Free Survival of >75%




The BMT CTN 0303 conclusions was that HCT following myeloablative preparative regimen for patients with AML in CR1 or CR2 can be performed in a multicenter setting using the CliniMACS® CD34 Reagent System without additional post-transplant pharmacologic GvHD prophylaxis
All 1º and most 2º endpoints were met, demonstrating:

- 81.8% Disease-Free Survival 6 months post TX
- No primary graft failure; consistent neutrophil and platelet engraftment
- Acute GvHD grades II-III <23%. No Grade IV aGvHD
- Chronic GvHD at 2 years 19%; extensive 6.8%
- TRM <20% at 2 years
- Overall risk of relapse was low at 23.7% at 2 years

The CliniMACS® CD34 Reagent System consistently produced a graft with > 2 x $10^6$ CD34+ cells/kg and < 1 x $10^5$ CD3+ cells/kg with no reported device related toxicities.

In January, 2014, the CliniMACS® CD34 Reagent System received FDA approval as a Humanitarian Use Device for processing hematopoietic progenitor cells collected by apheresis from an allogeneic, HLA-identical , sibling donor to obtain a CD34+ cell-enriched population for hematopoietic reconstitution following a myeloablative preparative regimen without the need for additional GvHD prophylaxis in acute myeloid leukemia.(44)

In summary, the use of CD34+ selected PBSC and manipulated bone marrow grafts without post-transplant GvHD prophylaxis for children was associated with rapid engraftment, low 100-day mortality, very low incidence of severe GvHD, and excellent survival. The overall survival compares favorably with matched-related and URD HSCT. Immune reconstitution was slow and post-transplant infections contributed to morbidity and mortality.

**2.2 Study Device Information**

**Miltenyi Biotec, Inc.**

**Brand: CliniMACS® CD34 Reagent System Information**

The CliniMACS® CD34 Reagent System is a medical device that is used in vitro to select and enrich specific cell populations.

The CliniMACS® CD34 Reagent System is comprised of four primary components:

- 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

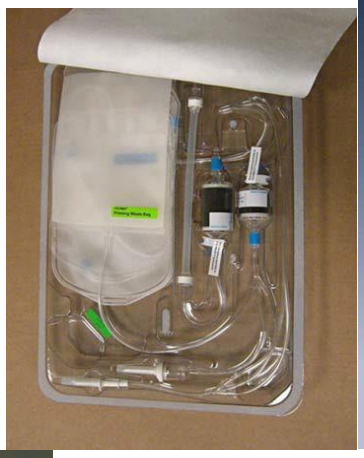
**Distributor:**
Corporate Headquarters Miltenyi Biotec Inc.
12740 Earhart Avenue
Auburn, CA 95602

**Manufacturer:**
Miltenyi Biotec GmbH, Clinical Products Friedrich-Ebert Strasse Technologiepark H-13
D51429
Bergisch Gladbach, Germany

**Manufacturer Contact:** Miltenyi Biotec Inc., Suite 305 120 Presidential Way
Woburn, MA 01801
Phone: (781) 782-1910
Fax: (781) 782-1920

CliniMACS® Tubing Sets (Standard and Large Scale) Standard: Up to 0.6 x $10^9$ CD34+ cells from up to 60 x $10^9$ Cells

Large Scale: 0.6-1.2 x $10^9$ CD34+ cells from 60-120 x $10^9$ Cells



CliniMACS® CD34 Reagent

CliniMACS® PBS/EDTA Buffer

## 2.3 Rationale

Cell dose is the most important factor affecting clinical outcomes in adult patients undergoing transplantation. Transplantation with a cell dose of less than 2.5 x $10^7$ nucleated cells per kilogram or a CD34 dose of less than 1.7 x $10^5$ per kilogram is associated with higher rates of non-engraftment, non-relapse mortality and lower survival (14). Alternative donors can provide ideal cell dose grafts and have the advantage of being rapidly available, but the higher probabilities of severe acute and extensive chronic GvHD due to HLA-mismatched is a significant source of morbidity and mortality. This study provides access to a model that potentially benefits from alternative NMDP and related donor pools to test the safety and efficacy of manipulated grafts using the CliniMACS® System. The aim is to ensure robust cell dose grafts with the best available donor for allogeneic HSCT recipients while providing a method for T cell depletion through CD34+ selection as the single form of GvHD prophylaxis. Currently, there is not a FDA-approved method for this process in the United States.

The outcomes in relatively small studies for children receiving unrelated donor transplants using the CliniMACS® have been comparable to or better than those receiving T cell depleted transplants with post-transplant immunosuppression (21, 25). This study will enroll up to 30 transplant recipients at Stanford. As the recommended prescription of allogeneic transplantation for different underlying diseases grows, enrollment on this protocol is expected to gradually increase in relation to the overall number of transplants performed in our center.

## 2.4 Study Design

This is a PI-initiated, single institution, open (no masking is used), prospective study to provide access to allogeneic graft manipulation through CliniMACS® System for qualifying candidates.

Currently, no FDA-approved method for T cell depletion exists. Recent experience with the CliniMACS® device has produced results with a 70-75% survival in children, many of whom were high-risk patients (19, 22, 36).

Patients will be enrolled with alternative (mismatched/haplocompatible) donors for an initial transplant.

The results with mismatched related or unrelated donor transplants so far justify expanding this approach to related donor recipients who have contraindications to post-transplant immunosuppression due to borderline organ function. Matched-related or unrelated donor recipients with borderline organ function will be allowed to enroll.

It is anticipated that the use of the CliniMACS® device will result in a very low risk of GvHD without the need for post-transplant immunosuppression.

This protocol will allow the use of patient-specific conditioning regimens that are available through open IRB studies in our institution or through open treatment plans from either the pediatric or adult stem cell transplant groups at Stanford University.

The follow-up schedule and affiliated data collection forms for the allogeneic recipients in this study are the standard CIBMTR data forms and procedures.
All recipients enrolled in the study and will be assessed for: neutrophil and platelet engraftment, graft rejection, serious infusion reaction, transmission of infection from HSCs infusion, EFS, TRM, acute and chronic GvHD, HSCT-related toxicities.

## 3. PARTICIPANT SELECTION AND ENROLLMENT PROCEDURES

Refer to the Participant Eligibility Checklist in Appendix A.

### 3.1 Inclusion Criteria

- Participants greater than one month of age.
- Participant has a disorder that can be treated with a hematopoietic stem cell transplant
- Participant's is eligible for an allogenic HSCT as per current institutional SOP and FACT standards.
- Participant must have a related or unrelated matched or mismatched-related donor who is:
- Able to receive granulocyte colony-stimulating factor (G-CSF) and undergo apheresis either through placement of catheters in antecubital veins or a temporary central venous catheter OR agrees on a bone marrow harvest;
- Healthy as per donor selection screening (following current SOP based on standards of foundation for accreditation of cellular therapy and stem cell transplantation – FACT);
- Willing to participate and sign consent.
- Participant or Legal Authorized Representative is able to sign informed consent (and signed assent, if applicable) for transplant.

### 3.2 Exclusion Criteria:

- Participant does not qualify for an allogeneic transplant due to medical screening, underlying disease, or lack of alternative donors.
- Any condition that compromises compliance with the procedures of this protocol, as judged by the principal investigator.

### 3.3 Informed Consent Process

All participants will be provided a consent form describing the experimental nature of the study with sufficient information for participants to make an informed decision regarding their participation.

The attending physician discusses the rationale, logistics, risks, and benefits of the study with the patient. Alternative therapies are discussed. The subject's bill of rights is reviewed. The recipient is given a copy of the consent form to take home and read. Then a meeting is scheduled for a consent conference to review the rationale, logistics, treatment plan, risks, benefits and consent form. The subject's bill of rights is reviewed with an emphasis placed on voluntary participation. Patients are given time to ask questions and do not need to sign the consent form until they are ready to proceed. In general, there is one hour devoted to the consent discussion with a clinical nurse specialist or research nurse educator. The attending physician is available to discuss the study with the patient at any time.

For pediatric patients, all efforts will be made to have both parents sign. If one parent is unable to sign, the reason will be documented in the consent note that is part of the patient permanent electronic medical records.

During the consent teaching, the physician, clinical nurse specialist or research nurse frequently asks questions of the subject to assess their understanding and a consent note is written documenting the patients understanding. For patients that are not fluent in English, the consent procedure is performed with an interpreter present (or via telephone), or a signer for those that are hearing impaired. Consents are translated into Spanish as needed. Patients are evaluated by a physician, nurse coordinator, clinical research nurse or clinical nurse specialist and social worker. Any member of the team can request a psychiatric evaluation if a concern of competency to provide consent is raised

### 3.4 Study Timeline

There will be no primary completion timeline to this study since the goal isto care for all HSCT recipients that need manipulated transplant graft. Subjects will continue to be followed on study until death or per institutional guidelines, whichever is longer.

## 4. PATIENT CONDITIONING, TRANSPLANT, AND POST-TRANSPLANT CARE

### 4.1 Patient Conditioning

Patient conditioning will be determined by disease and institutional guidelines for treatment.

### 4.2 Supportive Care Guidelines

Standard supportive care guidelines will be followed as per institutional standards and may vary with the condition or problems of the individual patients.

### 4.3 Criteria for Removal from Study

Conditions for individual patient termination:

- Disease progression
- Need for exclusionary concurrent treatment (per PI discretion)
- Withdrawal of informed consent
- Protocol non-compliance

The Principal Investigator may terminate the study for any of the following reasons:

- Significant toxicities related to the product graft
- If it becomes clear that the study treatment is less effective than otheravailable treatments.

### 4.4 Duration of Participation in the Study and Follow-up.

Patients will remain in the hospital until there is evidence of engraftment of donor cells as well as recovery from the side effects of transplant conditioning. When the patient has engrafted and meets institutional hospital discharge criteria, they will be discharged from the inpatient facility. Patients will be followed as outpatients at regular intervals in the Bass Center Outpatient Center in the Stem Cell Transplant Clinic. Duration of participation and follow up in study will conclude at day 100 post-transplant.

## 5 DONOR SELECTION AND PROCEDURES

Donors will undergo standard of care procedures for mobilization and leukapheresis. They will be asked to sign consents per standard of care procedures. There will be no research procedures for the donor.

### 5.1 Mobilization Therapy for Peripheral Blood Leukapheresis

Following screening and enrollment, the donor will receive mobilization therapy per institutional guidelines with daily G-CSF. Recommended dose of G-CSF is 16 mg/kg/day subcutaneously (rounded off to the nearest vial size of either 300 or 480 µg). Donors will receive G-CSF once a day for 5 days, with the leukapheresis occurring on day 5. If needed, an additional dose of G-CSF and leukapheresis procedure may occur on day 6 if needed to meet cell dose requirements. Plerixafor (mozibil) may also be given per institutional standards to increase peripheral blood stem cells prior to leukapheresis procedure.

Target post-selection cell dose is 20 x $10^6$ cells/kg with a CD3 cell dose of less than or equal to 3 x $10^4$ cells/kg.

### 5.2 Donor Follow-up

Donors will be followed per institution guidelines following FACT standards of care.

## 6 INVESTIGATIONAL PROCEDURE INFORMATION

### 6.1 Investigational Procedure

**CD34+ Selection**

CD34+ cell selection will be performed according to procedures given in the CliniMACS Users Operating Manual and institution SOPs. The processing will be performed at the Stanford BMT Cellular Therapeutics & Transplantation Laboratory at the Stanford University Medical Center.

The target cell doses will be ≥ 20 x $10^6$ CD34+ cells/kg. A minimum dose of ≥ 5 x $10^6$ CD34+ cells/kg will be acceptable. The target T cell dose will be ≤ 3 x$10^4$ CD3+ cells/kg.

The stem cells will be infused via central venous line into the recipient.

If > 20 x $10^6$ CD34+ cells/kg are available, then 20 x $10^6$/kg will be infused and the remainder will be cryopreserved in aliquots of 5 x $10^6$ CD34+ cells/kg.

Additional cell doses may be cryopreserved for future use.

### 6.2 Release of product for transplant

Sterility (routine USP culture for bacteria and fungi), endotoxin testing, gram stain, and viability studies will be performed in addition to the cell immunophenotyping. We already have experience with all of these procedures in other protocols at this institution (A Feasibility Trial of Post-Transplant Infusion of Allogeneic Regulatory T Cells Simultaneously with Allogeneic Conventional T Cells in Patients with Hematologic Malignancies undergoing Allogeneic Myeloablative Hematopoietic Cell Transplantation from Haploidentical Related Donors) and through pediatric stem cell transplant eIND and eIDEs submitted from this institution.

Viability, gram stain and endotoxin testing will be done prior to the release of the product for infusion. If the viability is ≥70%, the Gram stain is negative, and the CD34+ cell and CD3+ cell doses meet the criteria, the product will be released for infusion (target cell doses will be ≥ 20 x $10^6$ CD34+ cells/kg. The target T cell dose will be ≤ 3 x$10^4$ CD3+ cells/kg.)

If the culture becomes positive, appropriate antibiotics, per institutional protocols, will be administered to the recipient. The donor will undergo a clinical evaluation for infection and the reagents (including the aliquoted reagents) and procedures will be tested and reviewed to try to identify the source. If the gram stain is positive, the cells will be cryopreserved until the culture results are back, and the donor will undergo another leukapheresis providing he or she has no clinical evidence of infection.

**All donor apheresis collections will be assessed as follows:**

- Cell count by impedance counter (Coulter Z1)
- Viability by Propidium Iodide (PI) exclusion (flow cytometric assay) with target viability ≥70%,
- Sterility in accordance with 21 CFR 610.12 using Fluid Thioglycollate medium and Tryptic Soy Broth 14-day cultures
- Phenotypic analysis by flow cytometry for cell surface expression of CD34 and CD3

Final Products for infusion, including CD34 selected cells and CD3 fractions will be assessed prior to cryopreservation as follows:

a) Cell count by hemacytometer as per SCTT current SOP
b) Viability by Propidium Iodide (PI) exclusion (flow cytometric assay)
c) Sterility in accordance with 21 CFR 610.12 using FTM and TSB 14-day cultures as per current SCTT current SOP
d) Phenotypic analysis by flow cytometry for cell surface expression of CD34 and CD3

e) Endotoxin testing post-selection on each graft product as per SCTT current SOP

### 6.3 Agent Ordering

MACS Cell Separation Reagents are available through MACS and CliniMACS® Products

Miltenyi Biotec Inc.
2303 Lindbergh Street Auburn, CA, 95602, USA Phone: +1 530 888 8871
TOLL FREE: 800 FOR MACS
Fax: +1 530 888 8925
Email: macs@miltenyibiotec.com

- CliniMACS® plus Instruments are installed by qualified Miltenyi personnel
- Installation and Operational Qualification (IQ/OQ) is performed at time of installation
- All subsequent servicing and Preventative Maintenance are performed by qualified Miltenyi personnel
- Emergency Hotline Support

### 6.4 Agent Accountability

All quality validation will be checked at the Stanford BMT Cellular Therapeutics & Transplantation Laboratory at Stanford University Medical Center. The SCTT also has SOPs for cell sorting of PBSC and BM grafts to ensure consistent efficacy of the process. Cross reference letters are issued by the vendor, as per FDA guidelines, to notify dispensation of all supplies needed for the cell sorting.
All Reagents and materials are accounted for as per an approved Investigational New Drug (IND) application or Investigational Device Exemption (IDE) FDA guideline.

## 7 DOSE MODIFICATIONS

None anticipated. These are part of transplant issues but not specific to the use of the investigational device (CliniMACS® System).

## 8 ADVERSE EVENTS AND REPORTING PROCEDURES

### 8.1 Potential Adverse Events

Risk of contamination of the cell preparation with biologic or other foreign material. The sterility of system components that contact the cell sample and the detailed processing steps are designed to minimize potential contamination.

Significant animal and human studies have been done using these super-paramagnetic beads which are small in size (~50 nm in diameter) and are composed of iron oxide and dextran conjugated to murine monoclonal antibodies. These magnetic particles form a stable colloidal suspension and do not precipitate or aggregate in magnetic fields. The concentration of the conjugate is equivalent to 22 µg of antibody protein per ml of reagent, 800 µg/ml of dextran and 800 µg/ml of iron. Detailed toxicity studies have been undertaken to assess the safety of the antibody reagent when delivered to monkeys and rabbits in dosages significantly greater than the projected maximum dosage anticipated in clinical use (CliniMACS® Investigator brochure). There have been more than 100 separations for clinical use of the CliniMACS® system.

Reaction to CliniMACS® reagent - (murine monoclonal antibody is conjugated to an iron- dextran moiety. The iron dextran exposure from a single CliniMACS® separation is ~0.5 mg and less than 1 mg dextran.

The other reagent is the murine monoclonal antibody in which there is a risk of an anaphylactic reaction. The anti-CD34 monoclonal antibody, AC101 has been tested for safety in conformance with US standards. Systemic reactions appear related to the dose and rapidity of administration. Therapeutic levels (for cancer therapy or prevention of graft rejection) of mAb appear to be in the range of 2.5-5 mg/ml. The most commonly reported side effects have been myalgia, arthralgia, and flu-like symptoms. The CliniMACS® system results in the administration of a maximum of <15µg of antibody, 100x lower than therapeutic levels. Furthermore, studies have shown that the levels of antibody used in the CliniMACS® system do not induce complement activation in vitro.

## 8.2 Adverse Event Reporting

Recipients of allogeneic hematopoietic cell transplantation face significant risks. The risk of treatment related morbidity and mortality following allogeneic transplant is related to the underlying disease, disease status at the time of transplant, prior therapies and other coexisting health problems. Significant risks include:

- Infection. Risks include bone marrow suppression, prolonged hospitalization, invasive procedures, prior exposure to micro-organisms and immunosuppressive medications for the prevention and treatment of graft versus host disease. Infections may be caused by bacteria, viruses, protozoa or fungus.
- Graft versus host disease. Acute GvHD can range from a mild and treatable problem to a life threatening complication. Chronic GvHD can vary from a mild to an extensive disorder affecting almost any body tissue. Chronic GvHD can negatively affect functional status and quality of life.
- Graft failure. Although rare, graft failure is a fatal complication.
- Infertility in both genders and premature menopause in women at risk.
- Relapse. All patients face a risk of relapse. The risk is highest for those patients with active or resistant disease at the time of transplantation.

Definitions used for Reporting

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

**Life Threatening Adverse Event:** Any adverse event that, in the view of the investigator, places the patient at immediate risk of death from the reaction.

**Unexpected Adverse Event**: An adverse event, the nature or severity of which is not consistent with the applicable product information (Investigator's Brochure, product insert). For studies that do not involve investigational products or devices, an unexpected adverse event is an adverse event that is not described in the transplant medical literature or consent form.

**Serious Adverse Event (SAE):** Any adverse event occurring that results in any of the following outcomes: death, a life threatening adverse event, a persistent or significant disability/incapacity, a congenital anomaly, requires intervention to prevent permanent impairment or damage. Unless the Principal Investigator is using an investigational agent, s/he is not bound by the definition in Title 22 CFR. Stanford IRB definition(s) and requirements apply when applicable, Unanticipated Problems Involving Risks to Participants or Others (UPs) and Unanticipated Adverse Device Effect (UAD)

All grades 3-5 organ-related adverse events not due to the primary malignancy or pre-existing condition will be collected from day 0 through resolution of the SAE. Grade 4 infusion reactions will be reported expeditiously and as a separate listing in the FDA Annual Report.

**Distinction between Serious and Severe Adverse Events:** The term severe is used to describe the intensity (severity) of a specific event, for example mild, moderate or severe. The event itself however, may be of relatively minor medical significance, for example a severe headache. This is not the same as serious, which is based on the patient/event outcome and is usually associated with events that pose a threat to the patient's life or functioning. Seriousness, not severity, serves as a guide for defining regulatory obligations.

All Adverse events will be graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.3 found on the following website:

http://ctep.cancer.gov/reporting/ctc.html

The Principal Investigator (PI) or designee will assess each Adverse Event (AE) to determine whether it is expected or unexpected according to the Informed Consent, Protocol Document, or Investigator's Brochure; and its

relationship to the investigational product.

For the purpose of this study, only AE's found to be directly related to a study-mandated intervention will be captured. The following adverse events, if felt to be related to study treatment, will be captured, but not reported as SAEs:

- Hospitalization, prolongation of hospitalization, or escalation of care due to study-mandated intervention
- Disease Relapse
- Any problems with CliniMACS device during cell separation or inability to achieve CD34+ cell and CD3+ cell target doses
- Any positive culture of HSCT product
- Failure to engraft or graft rejection

All Serious Adverse Events (SAEs) will be tracked until resolution. SAEs will be collected from the time of stem cell infusion until 30 days post cell infusion. SAEs will be reported to the IRB if they meet the definition of expedited reporting per institutional regulations. All AE's and SAE will be reported at continuing renewal.

Reports (copy of faxed MedWatch) will also be sent to Miltenyi Biotec Incorporated, to the director of Regulatory Affairs and Safety Officer at (781) 782-1920.

Serious Adverse events will be reported on the MedWatch form 3500a when it meets the definition of expedited FDA reporting. All deaths will be examined to determine if they are related to the use of the CliniMACS device or infusion of manipulated product. If the death is determined to be related, the event leading to the death must be reported within 24 hours of the Investigator's awareness of the event.

The following SAEs require expedited reporting (as soon as possible but within at least 7 calendar days of the investigator learning of the event):

Deaths: All deaths up to 100 days post-transplant. This includes deaths from the common and expected grade 3-4 toxicities noted below. Deaths that occur outside of Stanford will be reported within 5 working days of knowledge of the death. It must be noted that obtaining detailed information on the cause and circumstances of a death occurring at another institution can be difficult. Excludes deaths related to relapse of underlying disease, which will reported in the annual report.

All serious and unexpected toxicities defined as those toxicities not identified in the transplant literature or in the consent form and that are felt to be related to protocol-mandated procedures.

**The following will not be reported as SAEs:**

- Hospitalizations: Approximately 50% of allogeneic transplant recipients will be readmitted to the hospital. The most common indications for readmission of an allogeneic HCT recipient are fever, failure to maintain nutritional status and graft versus host disease. Only hospitalizations felt to be related to study mandated interventions should be reported as SAE's.
- Relapse of disease: Disease relapse unfortunately remains a significant problem following both autologous and allogeneic transplantation. The risk of relapse is influenced by both patient and disease variables. The risk of relapse following allogeneic transplant is extremely dependent on the disease being treated but ranges from 10% (for patients with severe aplastic anemia) to 80% (for patients with refractory acute leukemia).
- Common and expected grade 3 - 4 toxicities of HSCT that are well described in the transplant literature, the product inserts or stated in the consent form and do not result in death. This includes but is not limited to neutropenia, thrombocytopenia, anemia, thrombotic microangiopathy, bleeding requiring transfusions, edema, hypertension, hypotension, gastritis, mucositis, nausea, vomiting, diarrhea, hematuria, central venous catheter infections, febrile episodes, sepsis, mental status changes, insomnia, mood alterations, seizures, tremor, pain, hypoxia, pleural effusion, pneumonitis, incontinence, infertility, laboratory abnormalities, veno-occlusive disease, graft failure, cardiac arrhythmias and graft versus host disease.
- Secondary Malignancies. The occurrence of secondary malignancies and associated mortality is a known risk of cancer therapies. The occurrence of secondary malignancies will be reported in the annual report.

# 9 DATA FORMS AND SUBMISSION SCHEDULE

Primary internal data monitoring will be performed by the Principal Investigator or designee. The PI will review data to assure the validity of data, as well as the safety of the subjects. The PI will also monitor the progress of the trial. The PI is responsible for maintaining the clinical protocol, reporting adverse events, assuring that consent is obtained and documented, reporting of unexpected outcomes, and reporting the status of the trial in the continuing renewal report submitted annually to the IRB and SRC. The PI may designate any or all of these tasks to qualified and trained staff.

Patient enrollment and toxicity/reporting will also be entered by the data-manager, coordinator, or designee into the CIBMTR and on the Program's REDCap database by the research staff.

# 10 MEASUREMENT

## 10.1 Primary and Secondary Outcome measures

The primary outcome will be severe (grade III/IV) acute graft-versus-host disease.

Secondary outcomes will be engraftment date (ANC>500 for 3 consecutive days, and >80% donor cells in blood), graft failure and graft rejection, immune recovery, infection, CMV infection and disease, EBV-related PTLD transplant-related toxicity and mortality, transplant-related mortality, grade 3/4 stem cell product infusion-related toxicity, relapse, DFS, OS.

## 10.2 Stopping Rules

The study includes stopping rules based on the incidence of graft failure (or graft rejection), and acute GVHD.

Stopping rule for primary graft failure: The first 30 enrolled patients (related and unrelated) will be evaluated for the stopping rule for primary graft failure. The trial is stopped if there are ≥ b graft failures out of k resolved patients. Only points where stopping is possible are listed.

| | | | | | | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *k* | 2 | 4 | 5 | 6 | 8 | 9 | 10 | 12 | 13 | 14 | 15 | 16 | 18 | 19 | 20 | 21 | 23 | 24 | 25 | 26 | 27 | 28 | 30 |
| *b* | 2 | 3 | 3 | 3 | 4 | 4 | 4 | 5 | 5 | 5 | 5 | 5 | 6 | 6 | 6 | 6 | 7 | 7 | 7 | 7 | 7 | 7 | 8 |

The stopping rule for graft failure yields the probability of stopping the trial of 0.05 if the rate of graft failure is 0.1. The probability of stopping the trial is 0.41 if the graft failure rate is 0.2, 0.82 if the graft failure rate is 0.3, and 0.96 if the graft failure rate is 0.4.

These probabilities were calculated based on the binomial distribution. The stopping rule was generated as described by Ivanova et al. (40).

If the study reaches a stopping boundary, the study will be suspended. At this point it may be terminated by the PI or submitted to the safety monitor with a description of the failures to date and a rationale for why the study should be continued. Proper use of the stopping rule table will be ensured by the Study Investigator.

Stopping rule for Grade ≥ IV toxicity: If any patient develops grade ≥ IV acute GvHD, grade ≥ IV infection, or any unexpected grade ≥ IV toxicity by 4 weeks post-transplant, the protocol will be halted and the processing re-evaluated before proceeding.

# 11 REGULATORY CONSIDERATIONS

## 11.1 Institutional Review of Protocol

The protocol, the proposed informed consent and all forms of participant information related to the study will be reviewed and approved by the Stanford IRB and other regulatory groups as necessary and required. Any changes made to the protocol will be submitted as a modification and will be approved by the IRB prior to implementation. The Protocol Director will disseminate the protocol amendment information to all participating investigators.

## 11.2 Data Management Plan

The PI, or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific Case Report Forms (CRFs) will document treatment outcomes for data analysis. Case report forms will be developed using the REDCap database system and will be maintained by the SCT clinical research nurse and/or program's CRA.

# 12 STATISTICAL CONSIDERATIONS

Interim descriptive analyses will be performed annually. As the eligibility for this protocol is very broad, analyses will be done separately for several pre-specified subgroups.

First, analyses will be conducted separately for patients transplanted for malignant diseases and those transplanted for non-malignant diseases. They will also be analyzed separately for transplants using mismatched related versus alternative donors. Finally, analyses will be conducted separately for primary vs. secondary allogeneic transplants. Further separation of the study population such as adults vs. pediatrics and myeloablative vs. non-myeloablative conditioning regimens will be considered depending on the patient numbers. In particular, neutrophil and platelet engraftment will only be analyzed using transplants with myeloablative conditioning regimens.

Baseline characteristics will be described using frequencies/percent or median/range as appropriate.

The study will enroll 30 transplant recipients at Stanford. As the recommended prescription of allogeneic transplantation for different underlying diseases grows, enrollment on this protocol is expected to gradually increase in relation to the overall number of transplants performed in our center.

# 13 REFERENCES

(1) Newburger P, Quesenberry P. Umbilical cord blood as a new and promising source of unrelated-donor

hematopoietic stem cells for transplantation. Current Opinion in Pediatrics 1996 February; 8(1):29-32.

(2) Balduzzi A, Gooley T, Anasetti C et al. Unrelated donor marrow transplantation in children. Blood 1995; 86:3247-56.

(3) CIBMTR Data. 2009.

(4) Wagner JE, Barker JN, Defor TE et al. Transplantation of unrelated donor umbilical cord blood in 102 patients with malignant and nonmalignant diseases: influence of CD34 cell dose and HLA disparity on treatment-related mortality and survival. Blood 2002 September 1; 100(5):1611-8.

(5) Rubinstein P, Carrier C, Scaradavou A et al. Outcomes among 562 recipients of placental- blood transplants from unrelated donors. N Engl J Med 1998 November 26;339(22):1565-77.

(6) Rocha V, Cornish J, Sievers EL et al. Comparison of outcomes of unrelated bone marrow and umbilical cord blood transplants in children with acute leukemia. Blood 2001 May 15;97(10):2962-71.

(7) Eapen M, Rubinstein P, Zhang MJ et al. Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. Lancet 2007 June 9;369(9577):1947-54.

(8) Cottler-Fox M, Cipolone K, Yu M, Berenson R, O'Shaughnessy J, Dunbar C. Positive selection of CD34+ hematopoietic cells using an immunoaffinity column results in T cell- depletion equivalent to elutriation. Exp Hematol 1995 April;23(4):320-2.

(9) Dreger P, Viehmann K, Steinmann J et al. G-CSF-mobilized peripheral blood progenitor cells for allogeneic transplantation: comparison of T cell depletion strategies using different CD34+ selection systems or CAMPATH-1. Exp Hematol 1995 February;23(2):147-54.

(10) Lemoli RM, Tazzari PL, Fortuna A et al. Positive selection of hematopoietic CD34+ stem cells provides 'indirect purging' of CD34+ lymphoid cells and the purging efficiency is increased by anti-CD2 and anti-CD30 immunotoxins. Bone Marrow Transplant 1994 April;13(4):465-71.

(11) McNiece I, Briddell R, Stoney G et al. Large-scale isolation of CD34+ cells using the Amgen cell selection device results in high levels of purity and recovery. J Hematother 1997 February;6(1):5-11.

(12) Elias AD, Ayash L, Anderson KC et al. Mobilization of peripheral blood progenitor cells by chemotherapy and granulocyte-macrophage colony-stimulating factor for hematologic support after high-dose intensification for breast cancer. Blood 1992 June 1;79(11):3036-44.

(13) Schmitz N, Dreger P, Suttorp M et al. Primary transplantation of allogeneic peripheral blood progenitor cells mobilized by filgrastim (granulocyte colony-stimulating factor). Blood 1995 March 15;85(6):1666-72.

(14) Korbling M, Huh YO, Durett A et al. Allogeneic blood stem cell transplantation: peripheralization and yield of donor-derived primitive hematopoietic progenitor cells (CD34+ Thy-1dim) and lymphoid subsets, and possible predictors of engraftment and graft-versus-host disease. Blood 1995 October 1;86(7):2842-8.

(15) Yeager A, Holand H, Mogul M. Transplantation of positively selected CD34+ cells from haploidentical parental donors for relapsed acute leukemia in children. Blood 1995;10, supplement:291a.

(16) Aversa F, Tabilio A, Terenzi A et al. Successful engraftment of T-cell-depleted haploidentical "three-loci" incompatible transplants in leukemia patients by addition of recombinant human granulocyte colony-stimulating factor-mobilized peripheral blood progenitor cells to bone marrow inoculum. Blood 1994 December 1;84(11):3948-55.

(17) Aversa F, Terenzi A, Tabilio A 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 May 20;23(15):3447-54.

(18) Handgretinger R, Klingebiel T, Lang P et al. Megadose transplantation of purified peripheral blood CD34(+) progenitor cells from HLA-mismatched parental donors in children. Bone Marrow Transplant 2001 April;27(8):777-83.
(19) Ortin M, Raj R, Kinning E, Williams M, Darbyshire P. Partially matched related donor peripheral blood progenitor cell transplantation in paediatric patients adding fludarbine and anti- lymphocyte gamma-globulin. Bone Marrow Transplant 2002;30:359- 66.

(20) Focused Workshop on Haploidentical Stem Cell Transplantation, Naples, Italy. 2004 Jul 8; 2004 p. 176-205.

(21) Lang P, Klingebiel T, Bader P et al. Transplantation of highly purified peripheral- blood CD34+ progenitor cells from related and unrelated donors in children with nonmalignant diseases. Bone Marrow Transplant 2004 January;33(1):25-32.

(22) Sleight B, Cowan M, Horn B, Jaroscak J, McGuirk J, Gilman A. Selected haplocompatible donor stem cell transplntation in children. Blood 2006;108:5417a.

(23) Ruggeri L, Capanni M, Urbani E et al. Effectiveness of donor natural killer cell alloreactivity in mismatched hematopoietic transplants. Science 2002 March 15;295(5562):2097-100.

(24) Leung W, Iyengar R, Turner V et al. Determinants of antileukemia effects of allogeneic NK cells. J Immunol 2004 January 1;172(1):644-50.

(25) Lang P, Handgretinger R, Niethammer D et al. Transplantation of highly purified CD34+ progenitor cells from unrelated donors in pediatric leukemia. Blood 2003 February 15;101(4):1630-6.

(26) Wagner JE, Thompson JS, Carter SL, Kernan NA. Effect of graft-versus-host disease prophylaxis on 3-year disease-free survival in recipients of unrelated donor bone marrow (T-cell Depletion Trial): a multi-centre, randomised phase II-III trial. Lancet 2005 August 27;366(9487):733-41.

(27) van Burik JA, Carter SL, Freifeld AG et al. Higher risk of cytomegalovirus and aspergillus infections in recipients of T cell-depleted unrelated bone marrow: analysis of infectious complications in patients treated with T cell depletion versus immunosuppressive therapy to prevent graft-versus-host disease. Biol Blood Marrow Transplant 2007 December;13(12):1487- 98.

(28) Eyearsich M, Lang P, Lal S et al. A prospective analysis of the pattern of immune reconstitution in a paediatric cohort following transplantation of positively selected human leucocyte antigen-disparate haematopoietic stem cells from parental donors. Br J Haematol 2001 August;114(2):422-32.

(29) Aversa F, Tabilio A, Velardi A. Transplantation of high risk acute leukemia with T cell depleted stem cells from related donor with one fully mismatched HLA haplotype. N Engl J Med 1998;339:1186.

(30) Dvorak CC, Gilman AL, Horn B, Cowan MJ. Primary graft failure after umbilical cord blood transplant rescued by parental haplocompatible stem cell transplantation. J Pediatr Hematol Oncol. 2009 Apr;31(4):300-3.

(31) Lacerda JF, Martins C, Carmo JA et al. Haploidentical stem cell transplantation with purified CD34 cells after a chemotherapy-alone conditioning regimen. Biol Blood Marrow Transplant 2003 October;9(10):633-42.

(32) Benaim E, Hale G, Horwitz E, Leung W. Reduced Intensity Conditionin (RIC) in haploidentical Transplantation. Blood 2004;104:2147a.

(33) Cooper N, Rao K, Gilmour K et al. Stem cell transplantation with reduced-intensity conditioning for hemophagocytic lymphohistiocytosis. Blood 2006 February 1;107(3):1233-6.

(34) Rossi G, Giorgiani G, Comoli P et al. Successful T-cell-depleted, related haploidentical peripheral blood stem cell transplantation in a patient with Fanconi anaemia using a fludarabine- based preparative regimen without

radiation. Bone Marrow Transplant 2003 March;31(6):437-40.

(35) Chaudhury S, Auerbach AD, Kernan NA et al. Fludarabine-based cytoreductive regimen and T-cell-depleted grafts from
alternative donors for the treatment of high-risk patients with Fanconi anaemia 1. Br J Haematol 2008 March;140(6):644-55.

(36) Hale G, Kasow K, Gan K, Horwitz E. Haploidentical stem cell transplantation with CD3 depleted mobilized peripheral blood stem cell grafts for children with hematologic malignancies. Blood 2009;106:2910a.

(37) Kramer JH, Crittenden MR, DeSantes K, Cowan MJ. Cognitive and adaptive behavior 1 and 3 years following bone marrow transplantation. Bone Marrow Transplant 1997 March;19(6):607-13.

(38) Craddock C, Szydlo RM, Klein JP, Dazzi F, et al. Estimating leukemia-free survival after allografting for chronic myeloid leukemia: a new method that takes into account patients who relapse and are restored to complete remission. Blood 2000 July;96(1):86- 90.

(39) Kaplan E, Meier P. Nonparametric estimation from incomplete observations. Journal of the American Statistical Association 1958;53:451-81.

(40) Ivanova A, Qaqish BF, Schell MJ. Continuous toxicity monitoring in phase II trials in oncology. Biometrics 2005 June;61(2):540-5.

(41) Przepiorka D, Weisdorf D, Martin P et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant 1995 June;15(6):825-8.

(42) Jacobsohn DA. Acute graft-versus-host disease in children. Bone Marrow Transplant 2008 January;41(2):215-21.

(43) Glucksberg H, Storb R, Fefer A et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. Transplantation 1974 October;18(4):295-304.

(44) U.S. food and Drug Administration/Center for Biologics Evaluation and Research.
January 23, 2014 approval Order-CliniMACS® CD34 Reagent System. 2014

(45) Lucarelli G, Polchi P, Izzi T, et al. Marrow transplantation for thalassemia after treatment with busulfan and cyclophosphamide. *Ann N Y Acad Sci* 1985;445:428-431.

(46) Parkman R. Bone marrow transplantation for genetic diseases. *Pediatr Ann*
1991;20:677-681.

(47) Shenoy S, Grossman WJ, DiPersio J, et al. A novel reduced-intensity stem cell transplant regimen for nonmalignant disorders. *Bone Marrow Transplant* 2005;35:345- 352.

(48) La Nasa G, Giardini C, Argiolu F, et al. Unrelated donor bone marrow transplantation for thalassemia: the effect of extended haplotypes. *Blood* 2002;99:4350- 4356.

(49) Jaing TH, Hung IJ, Yang CP, Lin TY, Chow R, Hsieh SI. Successful unrelated cord blood transplantation in a child with beta-thalassemia major. *J Trop Pediatr* 2005;51:122-124.

(50) Jaing TH, Yang CP, Hung IJ, Chen SH, Sun CF, Chow R. Transplantation of unrelated donor umbilical cord blood utilizing double-unit grafts for five teenagers with transfusion-dependent thalassemia. *Bone Marrow Transplant* 2007;40:307-311.

(51) Gennery AR, Slatter MA, Grandin L, et al. Transplantation of hematopoietic stem cells and long-term survival for primary immunodeficiencies in Europe: entering a new century, do we do better? *J Allergy Clin Immunol*

2010;126:602-610 e601-611.

(52) Bolanos-Meade J, Fuchs EJ, Luznik L, et al. HLA-haploidentical bone marrow transplantation with posttransplant cyclophosphamide expands the donor pool for patients with sickle cell disease. *Blood* 2012;120:4285-4291.

(53) Kamani NR, Walters MC, Carter S, et al. Unrelated donor cord blood transplantation for children with severe sickle cell disease: results of one cohort from the phase II study from the Blood and Marrow Transplant Clinical Trials Network (BMT CTN). *Biol Blood Marrow Transplant* 2012;18:1265-1272.

(54) Sodani P, Isgro A, Gaziev J, et al. T cell-depleted hla-haploidentical stem cell transplantation in thalassemia young patients. *Pediatr Rep* 2011;3 Suppl 2:e13

## APPENDIX A: Graft-Versus-Host Disease Grading

The modified Keystone criteria will be used for acute GvHD scoring for both pediatric and adult participants. See tables below.
If the criteria are not met but clinical signs and symptoms are consistent with GvHD and systemic therapy was begun, then the diagnosis of acute GvHD can be made. The staging of acute GvHD in the affected organ will be determined by the scoring team based on the severity of symptoms using the NIH Consensus Organ Scoring System of chronic GvHD, as a guide.
The onset date for acute GvHD will typically be the date when the systemic immunosuppression is added or escalated.
When acute GvHD progresses directly to chronic GVHD and a definite onset date cannot be determined, d+100 will be used for the onset date of chronic GvHD.
The diagnosis of acute or chronic GvHD will typically be based on clinical signs and symptoms and are not based on the time of symptoms after transplant or donor lymphocyte infusion (DLI).
The NIH Consensus recommendations will be used to establish the diagnosis of chronic GVHD:

Distinction from acute GvHD;
At least one diagnostic sign **OR** at least one distinctive sign confirmed by biopsy or other tests;
Exclusion of other diagnosis.
Chronic GvHD stated as suspected, presumed, and possible or not meeting the NIH consensus criteria be reviewed at the team's weekly patient meetings.

| Signs and Symptoms described as: | Chronic GvHD? |
|---|---|
| Inactive or Quiescent | No |
| Irreversible symptoms present | Yes |
| No symptoms, patient on GvHD therapy | No |

### Article I. Acute GvHD Grading

| Grade | Skin | Liver | Gut |
|---|---|---|---|
| 0 | None | None | None |
| 1 | Stages 1 - 2 | None | None |
| 2 | Stage 3, or | Stage 1, or | Stage 1 |
| 3 | | Stages 2 - 3, or | Stages 2 - 4 |
| 4* | Stage 4, or | Stage 4 | |

- Grade 4 may also include lesser organ involvement but with an extreme decrease in performance status.

**Article II. Acute GvHD Staging**

| Stage* | Skin† | Gut (adults)§¶ | Gut (children) §¶ | Liver‡ |
|---|---|---|---|---|
| 0 | No evidence of GvHD | < 500 mL/d diarrhea | < 10 mL/kg/d diarrhea | T.bili < 2.0 mg/dL |
| 1 | < 25% body surface area involved | 500 - 999 mL/d diarrhea, or persistent nausea with histologic evidence | 10 - 15 mL/kg/d diarrhea, or persistent nausea with histologic evidence | T.bili 2.0 - 3.0 mg/dL |
| 2 | 25% - 50% | 1000 - 1499 mL/d diarrhea | 16 - 20 mL/kg/d diarrhea | T.bili 3.1 - 6.0 mg/dL |
| 3 | > 50% | >/= 1500 mL/d diarrhea | 21 - 25 mL/kg/d diarrhea | T.bili 6.1 - 15.0 mg/dL |
| 4 | With bullous formation | Severe abdominal pain with/without ileus | > 26 mL/kg/d diarrhea | T.bili > 15.0 mg/dL |

- Criteria for grading minimum degree of organ involvement required conferring that grade.

† Use "rule of nines" or burn chart to determine extent of rash.
Range given as total bilirubin level.
§ Volume of diarrhea applies to adults. For pediatric patients, the volume of diarrhea should be based on body surface area.
¶ Persistent nausea with histologic evidence of GVHD in the stomach or the duodenum.

**Article III. Chronic Graft-versus-Host Disease Grading**

**ONSET:**

- De Novo – no prior aGVHD
- Interrupted – prior aGvHD resolved before onset of cGvHD
- Progressive – prior aGvHD did not resolve before onset of cGvHD

**GRADE:**

Limited – localized skin involvement and/or hepatic dysfunction due to cGvHD Extensive – one or more of the following:

- generalized skin involvement; or
- liver histology showing chronic aggressive hepatitis, bridging necrosis or cirrhosis; or
- involvement of eye: Schirmer test with < 5 mm wetting; or
- involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy; or
- involvement of any other target organ

**SEVERITY:**

- Mild – signs and symptoms of cGvHD do not interfere substantially with function and do not progress once appropriately t treated with local therapy or standard systemic therapy (corticosteroids and/or cyclosporine or FK 506)
- Moderate – signs and symptoms of cGvHD interfere somewhat with function despite appropriate therapy or are progressive through first line systemic therapy (corticosteroids and/or cyclosporine or FK 506)
- Severe – signs and symptoms of cGvHD limit function substantially despite appropriate therapy or are progressive through second line therapy

**APPENDIX B: Current Criteria Used at Stanford University at the Adult and Pediatric Programs Stem Cell Transplantation Programs to establish Graft Failure**