`An Expanded Access Study of the Feasibility of Using the CliniMACS® Device for CD34^{+} Cell Selection and T Cell Depletion for Graft-versus-Host Disease Prophylaxis in Alternative Donor Stem Cell Transplant Recipients

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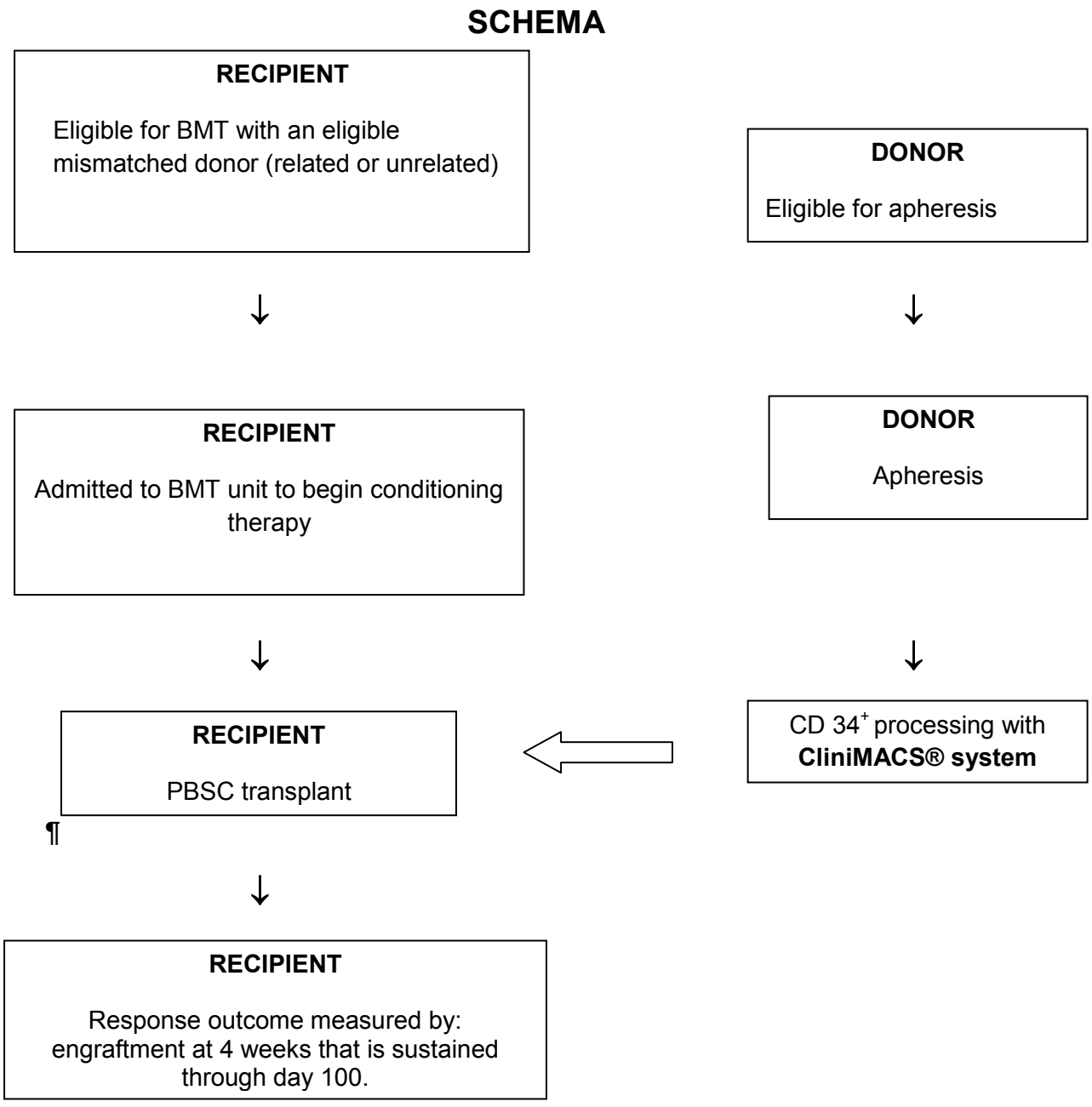
SCHEMA

Patient population: Patients with a malignant or non-malignant disease who can benefit from alternative stem cell transplantation, who lack a healthy HLA-identical related donor at least one year of age, and who have a mismatched related (≥ 4/8 and ≤ 7/8) or unrelated (6/8 or 7/8) donor able to receive G-CSF and undergo apheresis.

Study design:



LIST OF ABBREVIATIONS

AE	adverse event
ALL	acute lymphocytic leukemia
AML	acute myelocytic leukemia
ANC	absolute neutrophil count
APC	antigen presenting cells
ASCO	American Society for Clinical Oncology
BMT	bone marrow transplant
BSA	body surface area
CB	cord blood
CHR	Committee on Human Research (UCSF IRB)
CIBMTR	Center for International Blood and Marrow Transplant Research
CML	chronic myelogenous leukemia
CMV	cytometolovirus
CR	complete response
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
DFS	disease-free survival
DLI	donor lymphocyte infusion
DLT	dose limiting toxicity
EBV	Epstein - Barr virus
EFS	event-free survival
FA	Fanconi Anemia
FDA	Food and Drug Administration
FHCRC	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
G-CSF	human granulocyte-colony stimulating factor
GVHD	graft versus host disease
HLA	human leukocyte antigen
HSC	hematopoietic stem cell
ICH	International Conference on Harmonization
IDE	investigational device exemption
IEC	Independent Ethics Committee
IRB	institutional review board
JMML	juvenile myelomonocytic leukemia
KIR	killer cell immunoglobulin-like receptor
MDS	myelodysplastic syndrome
MRI	magnetic resonance imaging
MUD	matched unrelated donor
NMDP	National Marrow Donor Program
OS	overall survival
PBSC	peripheral blood stem cells
PCR	polymerase chain reaction
PI	Principal Investigator
PTLD	post-transplant lymphoproliferative disorder
PRC	Protocol Review Committee, UCSF HDFCCC
rATG	polyclonal rabbit anti-thymocyte globulin
SAE	serious adverse event
SOP	Standard Operating Procedure
TBI	total body irradiation
TMP/SMX	Trimethoprim-sulfamethoxazole
TRM	Transplant related mortality

SHORT ABSTRACT

A major issue in alternative donor (mismatched related and unrelated donor transplantation is the development of graft-versus-host disease (GVHD). Several clinical trials have shown that the use of T-cell depleted peripheral blood stem cells (PBSC) reduces GVHD in alternative donor transplants. The purpose of this study is to determine the ability of CD34 positive selection and T cell depletion using the CliniMACS® Device as the only GVHD prophylaxis to prevent severe acute GVHD in recipients of an alternative donor PBSC transplant. Mismatched related donors will match 4, 5, 6 or 7 out of 8 HLA antigens (haplocompatible) and unrelated donors will match 6 out of 8, or 7 out of 8 HLA antigens with the transplant recipient.
The patients will receive conditioning therapy based on their diagnosis which may include chemotherapy, anti-thymocyte globulin (ATG), +/- total body irradiation (TBI). The transplant recipients will be followed for 2 years post-transplant for the development of GVHD, engraftment, post-transplant infections, disease relapse, and overall survival.

1.0 GOALS AND OBJECTIVES

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 donor (mismatched related donor and unrelated donor) hematopoietic stem cell transplants.

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, and the rate of engraftment in recipients of CD34+ cell selected, T cell-depleted transplants from alternative 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) Leukemia relapse
h) Transplant-related mortality
i) Disease-free survival (DFS) and overall survival (OS)

Monitor device performance:

a) Purity of selected product
b) Yield of CD34+ cells
c) CD3+ cell depletion
d) Sterility

2.0 BACKGROUND AND RATIONALE

2.1 Stem cell transplantation

Stem cell transplantation (SCT) can cure some children with marrow failure syndromes, inherited immunodeficiencies, myelodysplastic syndrome (MDS), acute lymphocytic leukemia (ALL), acute myelogenous leukemia (AML), chronic myelogenous leukemia (CML), lymphoma, white blood cell disorders (chronic granulomatous disease, osteopetrosis), and red blood cell disorders (sickle cell disease, thalassemia). The preferred donor for transplantation is an HLA identical sibling. However, less than 20% of patients requiring a transplant will have such a donor. Alternative stem cell sources include volunteer matched unrelated donors (MUD) and unrelated donor cord blood (CB). Approximately 90% of Caucasians and 60% of African-Americans will have a 5/6 or 6/6-matched unrelated marrow donor. Approximately 80% of Caucasians and 40% of African-Americans will have a 5/6 or 6/6-matched unrelated cord blood donor. Most patients will have a 4/6-matched cord blood available (1).

The largest experience using unrelated donors is with unrelated donor bone marrow transplants. Single center data for children include a report from Fred Hutchinson Cancer Research Center (FHCRC) that described 47% 2 yr event-free survival (EFS) for ALL in CR1/2 and 46% for AML in CR1/2 (2). The incidence of severe, life-threatening (grade III-IV) acute GVHD was 37% and 62% of HLA-matched and mismatched recipients, respectively. Data from the Center for International Blood and Marrow Transplant Research (CIBMTR) from 1996-2001 shows a 50% 2 yr EFS for ALL in CR1 and 42% 2 yr EFS for ALL in CR2+. This compares to CIBMTR data for matched sibling transplants over the same time period that shows a 70% 2 yr EFS for ALL in CR1 and 60% 2 yr EFS for ALL in CR2+ (3).

For unrelated donor cord blood transplants, the University of Minnesota reported a 55% 2 yr EFS for ALL in CR1, 32% 2 yr EFS for ALL in CR2+, and 33% for AML in CR2+. The incidence of grade III-IV acute GVHD was 11%. The use of a 4/6-matched donor was associated with a relative risk of 2.4 for death compared to a 5/6 or 6/6-matched donor (p=0.01) (4). A larger review (562 patients) by Rubinstein et al. reported a 23% incidence of grade III-IV acute GVHD. The cumulative incidence of transplant-related events increased as the number of HLA disparities increased (5).

A comparison of MUD and CB donor sources in 541 children transplanted for acute leukemia showed a 2 year event-free survival of 43% for unmanipulated MUD transplant, 37% for T cell-depleted MUD transplant, and 31% for cord blood transplant. The incidence of grade III-IV acute GVHD was 29%, 8%, and 21% in the respective groups (6). A recent retrospective review of

MUD and CB donor transplant for 503 children with leukemia transplanted in the US showed the following results (7):

a) 2 yr EFS ~ 40% after matched and mismatched unrelated donor bone marrow transplant,
b) 2 yr EFS ~ 40% after 4/6 matched unrelated donor cord blood, ~ 45% after 5/6, ~ 65% after 6/6 matched (there were only 35 patients with a 6/6 matched donor),
c) Severe (Grade III/IV) acute GVHD 18% and 32% after matched and mismatched unrelated donor bone marrow transplant, respectively,
d) Severe (Grade III/IV) acute GVHD 27% after 4/6 matched unrelated donor cord blood, 20% after 5/6, 9% after 6/6 matched,
e) Relapse 39% and 31% after matched and mismatched unrelated donor bone marrow transplant, respectively,
f) Relapse 19% after 4/6 matched unrelated donor cord blood, 27% after 5/6, 31% after 6/6 matched.

2.2 Mismatched (haplocompatible) related donor stem cell transplant

Another donor source that has been reported is a mismatched (haplocompatible or sharing only one of two haplotypes) related donor. In the past, the major challenges have been engraftment, graft-versus-host disease (GVHD), and an increased incidence of infection and 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 order 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, and 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 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 100x lower than therapeutic levels. Following incubation, the product passes through a strong magnet. The CD34+ cells remain at the level of the magnet and other cells (T, B, and NK cells, monocytes, and neutrophils) pass through into a waste bag. The magnet is then turned off and the CD34+ cells are released into a bag containing the final product.

In addition, 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 the matched (13,14) or mismatched (15,16) relative. PBSC recruited with cytokines 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 a conditioning regimen of total body irradiation (TBI), thiotepa, fludarabine, and rabbit ATG for 101 patients. They used the CliniMACS® device to lower the T cell content of the donor PBSC to a median of 1 x 10^4/kg (range 0.04 – 3 x 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 2 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% 2 yr 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 mo. (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 yr. 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 mo. – 7 yrs. (median 31 mo.)(22). The median pt age was 8 yrs. (range 1-20). Patient with malignancy (n=13) included: AML - CR1 (primary induction failure, failed cord blood transplant) [1], CR2 [3]; MDS - RA/RARS [2], RAEB [2] AML (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 BMT 3 yrs 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. The conditioning regimen was TBI 12-14 Gy in 6 fractions, thiotepa, fludarabine, and ATG. Fractionated TBI was replaced with single fraction TBI (2 pts.) or melphalan (3 pts) as clinically indicated.

Cyclophosphamide was used instead of thiotepa for one pt with FA. No post-transplant graft-versus-host disease (GVHD) prophylaxis such as Tacrolimus or methotrexate was given. 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). Primary (occurring after SCT and prior to donor lymphocyte infusion [DLI]) 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 1 year transplant-related mortality were 11% and 19%, respectively. Four patients (of 13 at risk, 31%) have relapsed; 1 pt with cytogenetic relapse is in CR > 1 year later. The 2 yr 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 6 had HHV-6 reactivation. EBV reactivation occurred in 5/18 (28%) patients, 3 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 3 months post-transplant, only 4 of the 15 evaluable patients had a CD4 count > 100. By 9 months post-transplant, 10 of the 13 evaluable patients had a CD4 count > 200.

In summary, the use of megadose CD34+ selected PBSC without post-transplant GVHD prophylaxis for children was associated with rapid engraftment, a low 100-day mortality, a very low incidence of severe GVHD, and excellent survival. The overall survival compares favorably with MSD and MUD HSCT. Immune reconstitution was slow and post-transplant infections contributed to morbidity and mortality.

2.3 NK alloreactivity

The risk of relapse with haplocompatible transplantation may be reduced by utilizing both donor NK cells for a graft-versus-leukemia effect and donor lymphocyte infusions (DLI) to accelerate T cell reconstitution. NK alloreactivity has been shown to be present for myeloid leukemias and for lymphoid leukemias (23, 24). Alloreactivity can be determined by HLA-C typing with even better results with the addition of NK receptor typing (23, 24). In addition to HLA compatibility, the choice of the optimal donor source may depend on the age and size of the recipient, the type of leukemia, disease status, pre-transplant organ dysfunction, and pre-transplant infections.

There is limited experience with unrelated donor transplants for children following CD34+ cell selection and T cell depletion with the CliniMACS device (25). In 30 patients with leukemia, primary engraftment was observed in 84% with the remainder engrafted after a second transplant. Grade III-IV acute GVHD occurred in 7% of patients and followed HHV-6 infection in

both patients. The 2 year survival was 44% for patients in remission at the time of transplant (25). The same approach was used for unrelated donor transplantation for 14 children with non-malignant disease. Observed overall survival was 100% (follow-up 1-7 years) and no grade III-IV acute GVHD occurred (21).

In a large prospective study of T cell depletion versus immunosuppressive drugs for GVHD prophylaxis for adults undergoing matched unrelated donor (MUD) transplant, there was no difference in 3 year EFS, 27 vs 34%, respectively (26). However, the T cell depletion used for this trial was much less intense than that achieved with the CliniMACS® device, as demonstrated by an 18% incidence of grade III-IV acute GVHD (37% in the non-T cell-depleted arm) (26). Post-transplant infections including CMV and aspergillus were more problematic after T cell depletion (27). Interestingly, the outcome for recipients of T cell-depleted bone marrow who did not have a fungal infection was better than that for patients receiving T replete bone marrow. This suggests that successful prevention of post-transplant infection by hastening immune recovery may result in a superior outcome for T cell-depleted transplants.

2.4 Preliminary data

We have an ongoing study at UCSF 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 3×10^4/kg at the time of transplant. Seventeen evaluable patients have been enrolled with the following diagnoses: ALL (3), AML (6), bilineage 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%) are alive and well, 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. Of note is that 9/11 patients treated after the last protocol amendment are alive and well, including 5/7 with malignant disease. Both deaths were due to late infection. One patient died at 7 months after transplant due to Para influenza and Paecilomyces infections and the other died at 23 months after transplant due to disseminated Mucor infection. 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).

2.5 Immune reconstitution

A major challenge of previous approaches to haplocompatible donor transplantation is the prolonged immunodeficiency that follows transplant (28). This results in viral and fungal infections and is the primary cause of transplant-related mortality (29). As part of this study,

patients who have engrafted without evidence of GVHD will be eligible to receive donor lymphocyte infusions if clinically indicated (See section 7.5)

2.6 Rationale for including alternative conditioning regimens

Patients who are not eligible for the predecessor ongoing study described above include those who need an individualized conditioning regimen (ie. contraindication to the total body irradiation (TBI), fludarabine, thiotepa, and ATG regimen) or because of diseases/conditions not allowed in the eligibility criteria (e.g. non-Hodgkin's lymphoma, poor lung function) or who do not have a related haploidentical donor available or who have failed a prior transplant from a related or unrelated donor, e.g. unrelated cord blood transplant and who need a rescue transplant using an alternative donor. Examples of such patients that we have transplanted include (1) patient with AML who failed to engraft after a cord blood transplant and had invasive fungal infection – alive and well 4 years after transplant, and (2) patient with rare immunodeficiency – NEMO syndrome – who had significant lung dysfunction and was on oxygen at the time of transplant – alive and well 3 years after transplant, and (3) a patient with CD40 ligand deficiency who rejected a mismatched unrelated donor cord blood transplant and was successfully reconstituted with a paternal CD34+ T cell depleted haplocompatible transplant (30).

Recent studies using the CliniMACS® device, including our own, used a CD3+ cell (T cell) dose of $< 3 \times 10^4$/kg. Conditioning regimens include the TBI-containing regimen used by the Perugia group and in our study as well as non-TBI containing regimens as indicated by the patient's disease and clinical condition. The substitution of Melphalan for TBI has been reported by several groups (22, 31, and 32). Regimens have been developed for patients in poor clinical condition (32, 33) or with DNA repair syndromes (34,35). Because this protocol is designed to look at the ability of the CliniMACS® device to prevent GVHD, the conditioning regimen used will be chosen based on the patient's disease and clinical condition.

3.0 STUDY DESIGN AND ELIGIBILITY CRITERIA

3.1 Study design

Patients will be enrolled with alternative (mismatched/haplocompatible) related donors or unrelated donors either for an initial transplant or as a rescue following rejection of a previous graft or relapse following a previous transplant. For patients with mismatched related donors, the majority of clinical experience has been with a T cell-depleted PBSC product. Currently, no FDA-approved method for T cell depletion exists. Recent experience with the CliniMACS® device has produced excellent results with a 70-75% survival in children, many of whom were high risk patients (19, 22, and 36).

Patients that receive transplants from unrelated donors usually receive stem cells that are not T cell-depleted. However, this is associated with a high risk of GVHD. The excellent results with mismatched related donor transplants justify expanding this approach to unrelated donor

transplant recipients if the HLA mismatch is sufficiently great. 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. 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 replete transplants with post-transplant immunosuppression (21,25).

This protocol will allow the use of patient-specific conditioning regimens. Some patients have contraindications to certain components of the conditioning regimen used for our ongoing study under BB-IND 8817 (CC# 01151). An example is a patient with pre-existing organ dysfunction that would be better served by the use of a reduced intensity conditioning regimen. Another example is a patient for who total body irradiation is contraindicated due to very young age or prior radiation therapy. Finally, patients who would be otherwise eligible for the predecessor study but who do not have an eligible related donor or a closely matched unrelated donor would be eligible for this study. The target CD3+ T cell dose that will be given will be 3×10^4/kg. The UCSF 01151 protocol uses a dose of 3×10^4/kg. The T cell dose in the graft is usually $< 1 \times 10^4$/kg after processing and T cells are added to the product.

3.2 Recipient inclusion criteria

- Patient must have a malignant or non-malignant disease that can benefit from alternative stem cell transplantation according to standard practice guidelines for including patients for transplant as outlined in UCSF Pediatric BMT Standard Operating Procedure (SOP) *#206.04 Recipient Selection and Treatment Regimen for Pediatric Bone Marrow Transplant (PBMT).* Examples include acute and chronic leukemias, myelodysplastic syndrome, lymphoma, severe acquired and congenital cytopenias, white and red blood cell abnormalities, inborn errors of metabolism and immunodeficiencies. Patient with Fanconi's Anemia will be eligible regardless of match with donor.

- Patients with acute leukemia (AML excepted) or lymphoma must be in remission at the time of transplant.

- Patients must lack a healthy and willing HLA-identical related donor.

- If recipient is female and of child-bearing age, negative pregnancy test.
- Patient must either:
    - A) Have a mismatched related or an unrelated donor,
        - Mismatched related or unrelated donors must be:
            - Able to receive G-CSF +/- Plerixafor and undergo apheresis either through placement of catheters in antecubital veins or a temporary central venous catheter
            - Able to undergo Bone Marrow Harvest
            - Healthy and willing
            - See Donor selection criteria below
    - B) Be suitable for an autologous gene-modified transplant:

- using either bone marrow or cytokine-mobilized peripheral blood stem cells (PBSC)

- Patient or authorized guardian must sign informed consent for this study.

3.3 Recipient exclusion criteria

- Patient with an anticipated life expectancy of < 1 month
- Active infectious hepatitis or CMV disease (organ involvement)
- HIV or HTLV-I/II infection
- Cardiac ejection fraction < 45%; can be lower if patient is not in clinical cardiac failure and a reduced intensity conditioning regimen is used.
- Creatinine clearance <60 ml/min/1.72 m2; can be lower if a reduced intensity conditioning regimen is used.
- Pulmonary diffusion capacity (adjusted for Hgb), FEV1, or FVC <60% of predicted or O2 sat > 94% on room air if unable to perform PFTs; can be lower if a reduced intensity conditioning regimen is used.
- Serum ALT > 5 x upper limit of normal (can be up to 10x upper limit of normal if a reduced intensity conditioning regimen is used) or bilirubin > 2.
- Performance score (Lansky/Karnofsky) < 50
- Any condition that compromises compliance with the procedures of this protocol, as judged by the principal investigator.

3.4 Donor selection criteria:

Must have a complete medical history, physical and screening for infectious diseases that are acceptable for donation. See Section 7.1.2 for details of evaluation.
Donors must be willing to sign informed consent for this study. If donor is <18 years of age, donor must be willing to give assent and parents are willing to sign informed consent.

- 3.4.1 Related Donors
  - Sibling, half-sibling, parent, cousin, aunt, uncle or grandparent will all be considered eligible.
  - HLA antigen genotypic match ≥4/8 and ≤7/8 (haplocompatible).
  - If donor is female and of child-bearing age, negative pregnancy test if GCSF is to be administered.

- Criteria to consider when choosing among related haplo donors are:
    - CMV positive donor is preferred over other factors. CMV negative donor can be used ONLY if recipient is CMV negative.
    - HLA disparity i.e. 2 Ag mismatch preferred over 3 Ag mismatch; Drβ1 match preferred over class I match; HLA-C matched preferred over A and B match.
    - KIR mismatch in GVH direction is preferred for patients with malignant disorders
    - ABO compatibility
    - Absence of anti-HLA antibodies in recipient directed against donor antigens.

- 3.4.2 Unrelated Donors

    - 6/8 or 7/8 HLA antigens match (if two mismatches, they must be at different loci).
    - Absence of anti-HLA antibodies in recipient directed against donor antigens.
    - If donor is female and of child-bearing age, negative pregnancy test if GCSF is to be administered.
    - Must follow NMDP/Be The Match Algorithm Guidelines for Unrelated Donors below:
        - **NMDP: For unrelated donors: Per New Algorithm, Jan. 14, 2016**, effective immediately, NMDP/Be The Match is adopting a revised algorithm for determining if a donor is a research subject on their recipient's research protocol: The NMDP will inform the donor of the activities and the use of donor's apheresis product to be used in this study and obtain written consent from the donor. Transplant centers are sent documentation of the donor consent to participate in the research support activities. (The revised algorithm can be found on the "Be the Match Clinical Network")

- 3.4.3 Autologous Donors

    - If donor is female and of child-bearing age, negative pregnancy test if GCSF is to be administered.
    - Age ≥2 months for autologous bone marrow collection and CD34 selection (Gene Therapy Protocol, CHR# 16-18983).

3.5 Patient recruitment

Candidates are referred to study investigators from pediatric hematology/oncology specialists and immunologists. Patients and parents will be seen in the pediatric BMT outpatient clinic or as an inpatient where consent will be presented.

3.6 Patient registration

The patients will be registered by completion of eligibility forms and data entry into the UCSF Helen Diller Family Comprehensive Cancer Center Clinical Trial Management System (CTMS) and each patient will be assigned a unique subject study ID.

4.0 INVESTIGATIONAL TREATMENT PLAN

4.1 Dose

The plan is to use the CliniMACS® device to prevent severe (grade III/IV) acute GVHD without the need for post-transplant immunosuppressive medications. The target dose of CD34+ cells is ≥ $20x10^6$/kg, but a minimum of ≥$2x10^6$/kg is required. If the yield is <$2x10^6$/kg, we will attempt to obtain more donor cells (see Section 7.3). The target dose of CD3+ cells is $3x10^4$/kg. The ClinicMACS® CD34+ Reagent System will be used for donor $CD34^+$ cell selection. If we end up infusing <5x10^6 CD34/kg we will initiate a search for a back-up donor in the event the graft fails or is rejected and a second transplant is necessary. Target dosing for autologous gene therapy is defined in the Gene Therapy Protocol, *LVXSCID-ND: "A pilot feasibility study of gene transfer for x-linked Severe Combined Immunodeficiency in newly diagnosed infants using a self-inactivating lentiviral vector to transduce autologous CD34+ hematopoietic cells (CHR # 16-18983)".*

4.2 Duration of therapy

Patients will be followed for outcomes related to the device including acute and chronic GVHD and engraftment for 2 years. Subsequent follow-up care is standard for all transplant patients, and additional data related to secondary endpoints will be collected as available (see Appendix IV).

5.0 DOSE MODIFICATIONS AND TOXICITIES

These are part of related transplant-issues but not specific to the use of the investigational device (CliniMACS® device).

5.1 Recipient toxicity from the conditioning regimen [MC1]

5.1.1 Thiotepa

Risks of thiotepa include alopecia, mucositis, and hepatic toxicity. There is also cutaneous toxicity which can result in erythema and breakdown of the skin especially in the neck, axilla, and inguinal and perianal areas. This can be painful and can become secondarily infected. Significant skin toxicity can be prevented by frequent baths or showers when the drug is given. In combination with fludarabine and TBI the risk of fatal hepatic toxicity or fatal pulmonary toxicity is <5%. There has also been a multiorgan failure syndrome reported in association with thiotepa containing regimens. The incidence is low (<5%) and was seen when doses greater than 10 mg/kg were used.

5.1.2 Fludarabine

The primary toxicity of fludarabine in the doses used has been severe T and B cell immunodeficiency. It has also been associated with the development of immune mediated hemolytic anemias, unlikely to occur as part of an ablative conditioning regimen for BMT. Other side effects include nausea, vomiting and neurotoxicity (seizures, confusion). Neurotoxicity is very unlikely at the doses used on this study.

5.1.3 Total body irradiation (TBI)

The total TBI dose is 1200 cGy given in 6 fractions. All fractions will use 50% shielding of the lungs. Potential acute toxicities associated with the use of TBI include nausea, vomiting, and parotitis. Sub-acute complications or side effects of TBI include hair loss, mucositis, diarrhea, veno-occlusive disease, leukoencephalopathy and interstitial pneumonitis. Chronic complications include cataracts in about 20% of cases, delayed sexual development, short stature, and sterility in >95% of cases, and thyroid dysfunction in 5-10% of cases.

5.1.4 Rabbit anti-thymocyte globulin (rATG)

The most important risk associated with rATG is the development of a severe allergic reaction (i.e. anaphylaxis) which is rare. More common side effects of rabbit ATG include fever and rash.

5.1.5 Melphalan

Common side effects of melphalan include nausea, vomiting, low blood counts, mucositis, sterility, and hair loss. Much less common side effects include liver and lung damage.

5.1.6 Cyclophosphamide

Common side effects of cyclophosphamide include nausea, vomiting, low blood counts, sterility, and hair loss. Less common side effects include water retention, damage to the lining of the bladder leading to blood in the urine, and liver damage. Rare side effects include damage to the lungs and heart and secondary cancer (leukemia and lymphoma).

5.1.7 Busulfan

Common side effects of busulfan include nausea, vomiting, low blood counts, mucositis, sterility, temporary darkening of the skin, and hair loss. Less common side effects include liver damage. Rare side effects include lung injury and seizures (the latter are rare when prophylactic anti-convulsants are used).

5.1.8 Infection and bleeding

In general, due to the 1-2 weeks of neutropenia, thrombocytopenia and mucositis, there is a significantly increased risk of infection and bleeding. The mortality associated with these complications is generally <5%.

5.1.9 Mortality

The reported transplant related mortality (organ failure, infection, bleeding) is ~30% for patients with high-risk malignancies (25, 31, 32). In a limited number of children with non-malignant diseases the TRM was lower (10%) but until more patients in this category are evaluated, we will assume it is as high as 30%. For patients undergoing a transplant who have already failed a prior transplant (e.g. graft rejection post umbilical cord blood transplant) the TRM could be as high as 50%.

5.1.10 Secondary malignancy

There is also an overall reported risk of ~2-10% of a malignancy occurring up to 15 years post-transplant. The majority of these are lymphoproliferative disease related to EBV infection. Because these patients will not receive cyclosporine prophylaxis or post-transplant ATG, and because the processing procedure removes almost all B cells from the donor graft (the source of EBV in these circumstances), this risk is likely to be lower.

5.1.11 Decreased IQ

There is about a 30% risk of a decreased IQ (7 points) at 1 year post transplant (37). This occurs regardless of the conditioning regimen and by 3 years there appears to be some recovery of IQ points. Of the remaining patients, 1/3 has no change in their IQ and 1/3 increases their IQ by about 7 points.

5.1.12 Short stature

The risk of short stature post-transplant is related to the type of conditioning. With TBI the risk is highest (25%) while with busulfan containing regimens it is ~10%.

5.2 Recipient Toxicity from Transplant

5.2.1 Failure of engraftment

Published reports using 1-10x10^6 CD34+ cells/kg haplocompatible stem cell enriched T cell depleted PBSC with TBI-containing regimens have initial engraftment rates of >80% although with second transplants the engraftment rate has been >90%. We believe that the combination of thiotepa, fludarabine, ATG and TBI plus the large number of CD34+ cells in the graft should result in a primary engraftment rate of at least 80%.

5.2.2 Delayed T-cell reconstitution and increased infections

The chance of delayed T-cell reconstitution and increased infections is likely. In both children and adults who have been heavily pretreated it has taken as long as a year for T cell immunity (i.e. CD4+ cells > 200) to recover. The most commonly reported cause of transplant-related death with this kind of transplant is infection.

5.2.3 Increased risk of leukemic relapse

The extent of increased risk of leukemic relapse with PBSC is unknown. The studies of CD34+ PBSC haplocompatible transplants that have been reported to date do not demonstrate a higher than expected relapse rate.

5.2.4 Graft vs. Host Disease

In other studies in which $<7x10^4$ CD3+ cells/kg of haplocompatible PBSC are infused the incidence of grade III-IV acute GVHD has been <5%. In our current study using infusions up to $6x10^4$ CD3/kg we have seen an incidence of ~30% grade I-II acute GVHD and ~5% chronic GVHD. There have been no deaths due to GVHD.

5.3 Toxicity from Miltenyi Biotec Inc. CliniMACS® reagent system processing

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.
Paramagnetic microspheres - 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 300 separations for clinical use of the CliniMACS® system.

Reaction to CliniMACS® reagent - (murine monoclonal antibody conjugated to an iron-dextran moiety). Iron dextran is commercially available as a sterile solution of iron dextran complex for the treatment of severe iron-deficient syndromes. It contains 5% iron and 20% dextran, and its safety profile has been well characterized. Iron dextran solution contains 50 mg/ml of elemental iron, most of which is present in the ferric state. A total dose of iron-dextran for the average 70 kg person is calculated to be approximately 2 gm over several days (single dose of 100 mg). The iron dextran exposure from a singe CliniMACS® separation is ~0.5 mg and less than 1 mg dextran, 100x lower than a single dose and 1000x lower than a total dose.

CD34+ monoclonal antibody -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.

5.4 Donor PBSC toxicity

The toxicities listed below are of concern for all donors and not specific to this protocol or the use of the investigational device (CliniMACS® device).

5.4.1 G-CSF

For the recruitment of PBSC, G-CSF is known to cause bone pain in most patients as well as other symptoms including headache, bone discomfort or ache, ankle swelling or fluid retention. These symptoms are dose related and generally controlled with analgesics such as Tylenol or ibuprofen (17-19). There is at least a theoretical risk of inducing a malignancy with G-CSF although the extensive experience to date with normal donors does not indicate that this will be a problem. Patients with aplastic anemia or Kostmann's syndrome who have been chronically treated with G-CSF do not have any higher incidence of malignancy than is normally found with these disorders. Since G-CSF could theoretically interfere with embryogenesis, its use is contraindicated in pregnant women.

5.4.2 Plerixafor

Plerixafor has been used safely in a large number of patients and a limited number of healthy volunteers. In patients plerixafor was given in combination with G-CSF, the use of Plerixafor added to GCSF with GCSF dosing changed to 10 mg/kg/day x4 would be permitted as an alternative to the use of GCSFx8 or GCSFx4 without Plerixafor.
The most frequently occurring AEs (≥10%) were diarrhea, nausea, bone pain, fatigue, injection site reaction, headache, paraesthesia, back pain, hypokalemia, arthralgia, catheter site pain, vomiting, and dizziness. Also flatulence and insomnia (≥5%) have been rarely reported. The majority of patients experienced AEs that were mild or moderate and resolve within 24 hours**.** Systemic reactions (including urticaria, periorbital swelling, dyspnea, or hypoxia) and vasovagal reactions, orthostatic hypotension and/or syncope were seen in less than 1% of patients. The first studies with plerixafor in healthy volunteers and patients date from 2003 so follow-up is >10 years.

5.4.3 Apheresis

Apheresis has been associated with decreased platelets and temporary hypotension as well as hypocalcemia and some risk of bleeding because of anti-coagulation. There is also pain from the insertion of needles into the antecubital veins and the discomfort of a central line if necessary. All of these are reversible and have been tolerated in previously reported studies. There is the small risk (<1%) that a healthy donor might require a platelet transfusion. There is also the risk that peripheral venous access might be inadequate and placement of a temporary central line under local anesthesia will be necessary (17,18). The risks of inserting a central line include bleeding, infection and/or pneumothorax. There are abnormalities in the circulating white cell populations (T cells and stem cells) that occur after

apheresis in normal donors. Most of these appear to resolve (i.e. numbers return to normal) within the first 3 months post donation, but there may be abnormalities or long-term side effects that at this time have not been identified.

6.0 DEVICE INFORMATION

(Milteny Biotec, Inc. CD34+ ClinicMACS® reagent system information)

6.1 CD34+ cell processing

The collection will be stem cell enriched and T-cell depleted. The CD34+ cells are positively selected using the CliniMACS® System.

The pheresis product may be stored overnight at 4^{o}C and at a concentration < 200 x 10^6 cells/ml and processed the following morning. Products may be pooled (i.e. first and second collection pooled and third and fourth collection pooled) for processing and cell selection, or the collections may be processed and CD34+cells selected on each day. The determination of whether to store product overnight or select daily will be individualized per patient and be based on patient cell counts and CD34+ column cell capacity. CD34+ cell selection will be performed using the Miltenyi CliniMACS® system. The processing will be performed at University of California-San Francisco.

The target cell doses will be ≥ 20x10^6 CD34+ cells/kg . A dose of ≥10x10^6 CD34+ cells/kg will be acceptable. The target T cell dose will be 3x10^4 CD3+ cells/kg.

In the unlikely event that the CD3+ cell count is too high in order to achieve the minimum acceptable CD34+ cell dose, the patient can be given long course methotrexate or a course of cellcept along with a calcineurin inhibitor.

The stem cells will be injected intravenously into the recipient. Prophylactic Cefazolin* will be given for 24 hours beginning just prior to the infusion.

*Patients with penicillin allergy will be excluded from Cefazolin administration

If >35x10^6 CD34+ cells/kg are available, then 20x10^6/kg will be infused and the remainder will be cryopreserved. Otherwise, the full dose up to 35x10^6/kg will be infused.

Five aliquots of the negative fraction (non CD34+ selected cells) containing the CD3+ cells will be made and cryopreserved for future DLI. Each aliquot will contain a minimum of 10x10^4 CD3+ cells/kg body weight of the recipient.

Processing of samples received from participating centers:

Fresh harvested Hematopoietic progenitor cells **(**HPC**)** product are shipped to UCSF and then processed and cryopreserved and shipped back to the participating center for thawing and infusion.

6.2 Release of product for transplant

The studies that will be done in addition to the cell immunophenotyping will be sterility (routine USP culture for bacteria and fungi), endotoxin testing, gram stain, and viability. We already have experience with all of these procedures in other protocols, specifically, BB-IND 8817.

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.
If the culture becomes positive, appropriate antibiotics will be started. 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.

7.0 SCHEDULE OF ASSESSMENTS

(See Appendices II-III for Assessment Tables)

7.1 Screening assessments

7.1.1 Recipient pre-transplant evaluation (within 30 days of admission):

- Pulmonary function tests if patient age allows (usually > 5 years of age); if not, the patient will have pulse oximetry on room air.
- Echocardiogram and ECG
- CXR or chest CT scan (if prior history of lung disease)
- Creatinine clearance by Schwartz formula, urine collection, or GFR scan
- Testing for syphilis, CMV, HIV, EBV, HSV, VZV, Hepatitis B & C, HTLV I/II, and toxoplasmosis
- ABO typing and antibody screen
- Liver function tests, electrolytes, BUN, creatinine, CBC, differential.
- Marrow/Lumbar Puncture (LP) within 2 weeks of admission for patients with MDS/leukemia
- HLA typing
- Complete history and physical exam
- Evaluation for anti-HLA antibodies against donor antigens
- Pregnancy test if female and of childbearing age.

7.1.2 Donor pre-apheresis evaluation (must be done within 30 days of donation) (related donors only):

Complete history will include:

- Surgical/anesthesia history
- Review of systems
- History of inherited conditions and chronic illness
- History of hematological problems and immunological disorders
- History of cancer
- Donor health questionnaire which includes transfusion, vaccination and travel history
- List of current medications and allergies.
- Screen for evidence of syphilis, CMV, HIV, EBV, Hepatitis B & C, HTLVI/II, West Nile virus, Chagas' disease, and toxoplasmosis
- ABO typing and antibody screen
- Liver function tests, electrolytes, BUN, creatinine, CBC, diff, PT, PTT
- HLA typing
- ECG
- Chest x-ray
- Pregnancy test if female and of child-bearing age

Donor eligibility will be determined in accordance with 21 CFR 1271.45-.90 and the UCSF BMT Program Hematopoietic Progenitor Cell Donor and Recipient Evaluation Guidelines.

Unrelated donor product will be received via the National Marrow Donor Program (NMDP). Donors will have been evaluated per NMDP standard protocols.

7.2 Therapy

7.2.1 Recipient cytoreductive regimen - this will be patient-specific.

The standard regimen for leukemia will be:

- Total Body Irradiation (TBI) followed by chemotherapy (see table below):

Day -9	200 cGy TBI x 2
Day -8	200 cGy TBI x 2
Day -7	200 cGy TBI x 2
Day -6	Fludarabine 40 mg/m^2 (1.33 mg/kg if ≤ 12 kg body weight) Thiotepa 10 mg/kg/day divided into 2 doses 12 hours apart
Day -5	Fludarabine 40 mg/m^2;
Day -4	Fludarabine 40 mg/m^2;
Day -3	Fludarabine 40 mg/m^2; Rabbit ATG 0.5 mg/kg
Day -2	Fludarabine 40 mg/m^2; Rabbit ATG 1.5 mg/kg
Day -1	Rabbit ATG 1.5 mg/kg
Day 0	Transplant

Example of other conditioning regimens include (see Appendix I for details):

- Substitution of melphalan 140 mg/m2 for TBI especially for non-malignant, non SCID diseases.
- Substitution of busulfan and melphalan for TBI and thiotepa
- Substitution of cyclophosphamide for thiotepa in patients with Fanconi anemia

These modifications will be based upon published conditioning regimens (22,31-35).

- The autologous gene therapy conditioning uses targeted busulfan at a low dose exposure that is specified in the gene therapy protocol.

Patients with severe combined immunodeficiency and lack of NK cells or function and no GVHD due to maternal engraftment may be transplanted without conditioning.

Total dose of the TBI (1200 cGy) will be delivered in 200 cGy fractions separated by at least 6 hours. The patient will be treated with AP and PA fields and lungs will be partially blocked such that the dose to the lungs will be 600cGy **(50% clinical shielding of lungs). For patients with MDS/leukemia, there will be an extra boost of radiation to the ribs).** Thiotepa will be given IV on day –6 at a dose of 5 mg/kg every 12 hrs x 2. Each infusion will be given over 4 hrs. Very careful attention to skin care during thiotepa treatment is necessary. The patient should shower (or bath for small children) at least three times daily during administration and for 24 hrs after the last dose of thiotepa.

Fludarabine will be administered each day, from–6 to–2, at a dose of 40 mg/m^2 as a 30 minute infusion. The fludarabine dose will be reduced by 20% for patients with creatinine clearance ≤70 ml/min/1.73m^2.

Rabbit ATG will be infused each day, from –3to –1 as 10 hour infusion.

7.2.2 GVHD prophylaxis and therapy

There will be <u>no</u> post-transplant GVHD prophylaxis given unless the CD3+ cell dose is too high (see Section 6.1).

Initial treatment of GVHD will be prednisone or methylprednisolone 1-2 mg/kg/d. A rapid taper can be used if there is a quick and complete response. Additional therapy may be added if needed.

7.2.3 Infection prophylaxis and pre-emptive therapy

The infection prophylaxis listed below can be modified by the principal investigator if clinically necessary (this includes changing drugs and time period as clinically relevant).

Herpes viruses prophylaxis:

a) acyclovir 250 mg/m^2/dose IV q8h until discharge then PO per standard supportive care guidelines until the CD4>200 and PHA response ≥30% of lower normal control.

CMV:
All recipients will have CMV testing by PCR once per week until day +100 after transplant and then q2 weeks until CD4>200/uL. Monitoring should be more frequent in patients who have prolonged CMV reactivation after transplant. If the PCR test becomes positive, ganciclovir will be started at an induction dose of 5 mg/kg IV q12 x 7-14 days, then a maintenance dose of 5 mg/kg/day 5-7 days per week until PCR is negative x 2 weeks (assuming the patient remains asymptomatic). Valganciclovir can be used as prophylaxis following ganciclovir therapy if CMV reactivation occurred when the patient was on acyclovir prophylaxis. Alternatively, patients may be treated with foscarnet induction and maintenance. If CMV PCR does not clear with ganciclovir therapy or if drug resistance develops, DLI should be considered. Patient may also be eligible for CMV-specific cytotoxic T cell study.

EBV:
EBV by PCR will be obtained weekly beginning at 1 month post SCT and continuing until day +100 after SCT then q2wks until CD4>200. Monitoring should be more frequent in patients who have EBV reactivation after transplant. If the EBV viral load is > 1000 copies/ml or if any level of positivity is present with fever and/or other clinical findings such as adenopathy, rituximab 375 mg/m2 IV weekly x 2-4 will be given. CT scan of head, neck, chest, abdomen, and pelvis should be considered to evaluate for adenopathy. A therapeutic donor lymphocyte infusion can also be considered.

HHV-6:
HHV-6 PCR will be obtained weekly until day +60 after transplant and then q2wks until day +100.

Adenovirus:
Adenovirus PCR will be obtained on patients with fevers and negative bacterial/fungal blood cultures. For adenovirus reactivation (positive PCR), cidofovir will be given. This will be given as 5 mg/kg weekly or 1 mg/kg three days per week until the PCR is negative.

Pneumocystis carinii prophylaxis:
Trimethoprim/sulfamethoxazole 5 mg/kg/day of TMP component divided BID for 3 consecutive days per week. If TMP/SMX is not tolerated, alternative prophylaxis will be given per institutional policy.

Fungal prophylaxis:
Fluconozole 3 mg/kg (max 200 mg) PO (or IV if PO not tolerated) qd will be started on Day +1 and continued until CD4 > 200 and PHA ≥30% lower limit of normal control.

Bacterial prophylaxis:
None

IVIG:
Gammaglobulin will be administered at a dose of 200 mg/kg every 2 weeks while hospitalized and then 400 mg/kg every 4 weeks until IgM ≥ 50 AND isohemaglutinin titers are ≥1:8.

7.2.4 Supportive care

No post-transplant G-CSF unless the ANC is <500 on day +14; G-CSF may be started as early as Day +6 in patients with active fungal infections at the discretion of the treating physician.

All transfusions (except the stem cell product) will be irradiated and leukodepleted. In addition, CMV negative PRBC will be used.

Direct and indirect Coombs test will be checked q 2 weeks beginning at 8 weeks post BMT until the CD4>200 and PHA≥30% of the lower limit of normal control because of the risk of autoimmune hemolytic anemia.

7.3 Mobilization and collection of donor peripheral blood stem cells

The donor will receive 4 days of granulocyte colony stimulating factor (G-CSF, Neupogen®) administered subcutaneously (5 µg/kg/dose) twice a day. The total daily dose may be reduced to 10 mcg/kg and the dose may be given once a day for an unrelated donor if the recommended dosing is not possible. When determined to be clinically necessary by the treatment team, Plerixafor 240 mcg/kg will be administered subcutaneously approximately 9 hours prior to planned collection. This will generally be required for donors of patients > 15 kg, where historically a 2nd day collection has almost always been required. The donor will undergo an outpatient apheresis using antecubital veins for venous access if possible. If the peripheral venous access is inadequate, a temporary central line will be placed as an outpatient. The morning dose of G-CSF will be omitted on the day of collection if apheresis will take place in the morning. G-CSF will be continued until the completion of apheresis. If the donor's white blood count determined on the 4th day and subsequent days of G-CSF (for patients receiving 5 mcg/kg/dose q12) is greater than 60 x 10^9 cells/L, the G-CSF dose on the following day should be decreased to 3ug/kg/day.

For the apheresis, 5-20 x 10^{10} mononuclear cells (depending on the recipient's body weight and efficacy of CD34+ cell mobilization in the donor) will be collected which may require 1-3 apheresis procedures. Additional days of collection will require additional doses of G-CSF and possibly Plerixafor. Each pheresis procedure will process 5 blood volumes (max 25 L) and will be performed in accordance with the UCSF BMT Program Pediatric PBSC Mobilization and Collection Guidelines.

In the unlikely event the yield of CD34+ cells is too low or the product is not deemed acceptable for infusion, a related donor may be mobilized again (probably using Plerixafor (AMD3100) or another relative may be asked to act as an alternative donor. Unrelated donors may be asked for a second donation or another unrelated donor will be sought. It is our standard policy with unrelated donor transplants to identify an alternative if the first donor doesn't engraft. We would do the same for this protocol. Each occurrence of a transplant procedure will use cellular product from only one donor.

7.4 Treatment of graft failure

If at 4 weeks post-transplant there is no evidence of engraftment based upon blood counts, bone marrow examination and chimerism assays (<5% donor cells), infusion of a second PBSC transplant will be offered using the same or another donor (the other parent, sibling, or unrelated donor). Additional chemotherapy and immunosuppression will be given prior to the second transplant as needed. If pancytopenia occurs at a later time point, the same approach may be used. Post-transplant endpoints for the second transplant will not be included in study analyses.

7.5 Therapeutic donor lymphocyte infusion (DLI)

Indication:

- Mixed Chimerism (< 80% donor) or increasing recipient chimerism
- Relapse
- EBV-related PTLD
- Viral infections that progress despite treatment with anti-virals
- In adequate T cell reconstitution
    - CD4<100 on day +100
    - PHA<10% of normal control level on day +100

7.5.1 Collection of therapeutic donor lymphocytes:

DLI will usually be obtained from the cryopreserved negative fraction (T cell-containing) of the CD34+ cell selected G-CSF-mobilized PBSC.

T cells from the G-CSF-mobilized PBSC may be less alloreactive than T cells collected from blood when G-CSF is not present. G-CSF increases Th2 cells that decrease IL-12 production and delays immune recovery. At the discretion of the treating physician, a new collection can be performed to provide DLI depending on the clinical situation (such as leukemic relapse). For larger recipients, the stem cell donor will undergo lymphocytopheresis to collect CD3+ cells in aliquots of 5 X 10^4/kg, 1 X 10^5/kg, 5 X10^5/kg, and 10^6/kg. For small children requiring small doses, donors may give whole blood if ABO compatibility permits the use of whole blood as a source of DLI. All additional CD3 collections, whether from a blood draw or an apheresis will be GCSF mobilized comparable to the initial mobilization for the transplant.

DLI will not be given if the patient has active GVHD and/or is on immunosuppressive therapy for previously active GVHD.

7.5.2 DLI Dose

The dose of DLI ($3x10^4$ – $1x10^5$ CD3/kg) will be determined by the treating physician based on the clinical situation.

7.6 Follow-up assessments

Follow-up studies which are standard for all transplant patients will be performed according to BMT guidelines and include assessment of engraftment, marrow function, immune status, liver and kidney function, pulmonary function tests, and echocardiogram. (See Appendix IV)

8.0 CRITERIA FOR TERMINATION

8.1 Conditions for terminating the study

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

- Significant toxicities
- If it becomes clear that the study treatment is less effective than other available treatments.

8.2 Conditions for individual patient termination

The Principal Investigator may terminate the participation of an individual patient for any of the following reasons:

- Disease progression
- Need for exclusionary concurrent treatment
- Withdrawal of informed consent
- Protocol non-compliance
- Lost to follow-up

9.0 STATISTICAL CONSIDERATIONS

9.1 Hypotheses

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%. This has been our experience in 18 reported patients (22) and currently 25 treated recipients of haplocompatible donor transplants.

9.2 Sample Size

Patients will be enrolled into one of two cohorts, haplocompatible related donor (n=60) and unrelated donor (n=20). The study objective is to determine the ability of CD34+ selection using

the CliniMACS® device as the sole GVHD prophylaxis to prevent severe (grade III-IV) acute GVHD by Day +100 after transplant in recipients of alternative donor stem cell transplants. It is anticipated that the incidence of GVHD will not be affected by whether or not patient has had a previous transplant. Based on the literature and our experience, graft rejection is expected to occur in < 10% of patients. Sixty patients with related donors will allow estimating the proportion of severe acute GVHD with the exact 95% confidence interval with a maximum total width of 0.168 if the observed rate of severe acute GVHD does not exceed 10%. If a maximum occurrence of 6 severe acute GVHD events is observed in 60 patients, the exact 95% confidence interval is (0.04, 0.21). For 20 patients with unrelated donors the maximum width of the exact 95% confidence interval for a 10% maximum occurrence of GVHD is 0.305. A very low incidence of severe GVHD is predicted based on a reported incidence of 2% in a large study of adults (17) and a 0% incidence in a large study in children (21) and our experience (22).

The maximum total width of the exact 95% confidence interval is displayed below for an observed proportion of severe acute GVHD not exceeding 10% based upon the total number of evaluable patients:

Related (n=60)		Unrelated (n=20)	
# Evaluable Pts.	Total Width for the Exact 95% CI	# Evaluable Pts.	Total Width for the Exact 95% CI
60	0.168	20	0.305
29	0.159	19	0.259
28	0.161	18	0.272
27	0.164	17	0.286
26	0.166	16	0.300
25	0.169	15	0.317

9.3 Endpoint definitions

1. GVHD: Grading of GVHD will be according to Appendix V and VI

2. Engraftment: Primary graft failure (lack of engraftment) will be the lack of recovery of ANC to > 500 by Day +28 after transplant in the absence of immunological graft rejection. This endpoint is intended to monitor graft quality after manipulation.

3. Graft rejection:

    a. Initial evidence for marrow recovery and engraftment with subsequent pancytopenia without another cause (i.e. Infection and/or drug therapy)

    b. Decrease in chimerism greater than 50% from highest level achieved

4. Chimerism at Day 100: Chimerism (percentage of donor cells) will be assessed at Day 100 post-transplant in evaluable (without disease progression) patients.

5. Immune recovery: Immune recovery will be assessed by the time to CD4>100 and CD4>200 and the PHA to ≥10 and 30% of lower limit of normal.

6. Severe toxicity: Severe toxicity will include Grade 3 unexpected (for transplant recipient) toxicity or any non-hematological grade 4 toxicity.

7. Post-transplant infections: Infections that occur following the initiation of the conditioning regimen.

8. CMV infection and disease: CMV infection will be reactivation detected by CMV PCR on plasma and CMV disease will be evidence of organ involvement (eg. CMV pneumonia, enteritis).

9. Post-transplant lymphoproliferative disease (PTLD): PTLD will include patients that have clinical PTLD and those with EBV viral load > 1000 copies/mL.

10. Transplant-related mortality (TRM): TRM will include death due to regimen-related toxicity or GVHD (usually all causes other than disease relapse or when infection is present at enrollment).

11. Disease free survival (DFS) and overall survival (OS): Disease-free survival will be survival without relapse, including molecular, cytogenetic, and morphological relapse. DFS and OS at one and two years will be determined from the day of transplant. DFS and OS at 5 years after transplant, pending availability of clinical follow-up data, will be determined from the day of transplant.

12. Device (CliniMACS®) performance parameters: The parameters will include the purity of the CD34+ selected stem cell product, yield of CD34+ cells after selection, degree of CD3+ cell depletion after selection, and viability and sterility of the stem cell product after selection.

9.4 Plan of analysis

The primary endpoint of the study is the incidence of severe (grade III/IV) acute GVHD occurring by Day +100 after transplant. This will be assessed for each cohort separately (related and unrelated donor). In our experience, almost all patients who develop acute GVHD after this type of transplant do so by Day +30.

The following variables will be assessed as secondary endpoints with results reported for each study cohort. Frequencies of secondary endpoints will be summarized by proportions with 95%

confidence intervals. Percentages will be summarized by mean values with 95% confidence intervals. Durations will be measured from the date of transplant:

a) Engraftment: The incidence of primary graft failure and graft rejection will be determined.

b) Chimerism at Day 100: The percentage of donor cells will be reported for all evaluable (without disease progression) patients.

c) Immune recovery: The time to CD4 count >100 and > 200 and PHA≥10% and 30% will be calculated.

d) Severe toxicities: The incidence of severe toxicities will be determined. This will include grade 3/4 stem cell product infusion-related toxicity.

e) Post-transplant infections: Post-transplant infections will be described by incidence and type.

f) CMV infection and disease: The incidence of each will be calculated.

g) Post-transplant lymphoproliferative disease (PTLD): The incidence of PTLD will be calculated.

h) Transplant-related mortality (TRM): The incidence of TRM will be calculated at Day 100 after transplant and long term.

i) Disease-free survival: The method of Craddock et al. (38) will be used to estimate DFS. The definition of DFS will parallel that of leukemia-free survival proposed by Craddock.

j) Overall survival: Overall survival will be estimated using the Kaplan-Meier method (38).

k) Device (CliniMACS®) performance parameters: The parameters will be summarized for all products that are processed and median and ranges will be determined.

The endpoints will be monitored at least annually. The frequency will depend on the number of patients enrolled.

9.5 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_k 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.

9.6 Accrual

Patients will be recruited by the PI and co-investigators. A total of 80 patients will be enrolled into the study: 30 patients into an alternative related donor cohort and 20 patients into an unrelated donor cohort.

9.7 Estimated duration of study

The study will require 5 years for accrual and 2-5 additional years for follow-up studies. In addition, DFS and OS will be further analyzed using available data from standard clinical follow-up at 3-years, 4-years, and 5-years post-transplant.

9.8 Replacement policy

Enrolled patients who fail to complete the study will not be replaced. If a patient dies prior to evaluation for engraftment, the patient will not be included in analysis of the primary outcome (see Section 9.2).

10.0 CRITERIA FOR EVALUATION

10.1 Monitored outcomes

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

Secondary outcomes will be engraftment (ANC>500 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.

If a second PBSC transplant is offered as described in Section 7.4, post-second-transplant outcomes will not be included in study analyses.

11.0 DATA AND SAFETY MONITORING PLAN

11.1 Oversight and monitoring plan

The UCSF-CCC Data Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and patient safety for all UCSF-CCC institutional clinical studies.
A summary of DSMC activities for this study includes:

- Review of subject data
- Review of all serious adverse events
- Minimum of a yearly audit

Data Safety Monitoring Committee Contacts:

DSMC Chair:	Alan Venook, MD
Phone:	(415) 353-2745
Email:	venook@cc.ucsf.edu
Box:	1705

DSMC Monitors:
Box: 1297

11.2 Monitoring and reporting guidelines

Investigators will conduct continuous review of data and patient safety at weekly study group or site committee meetings where the results of each patient's treatment are discussed. The discussion will be documented in the minutes and made available to the DSMC as requested. The discussion will include number of patients, significant toxicities as described in the protocol, and observed responses. All grade 3-5 **unexpected** adverse events (AE) and serious adverse events (SAE) related to study participation will be reported to the FDA, device manufacturer, CHR, DSMC and Miltenyi Biotec, Inc.

11.3 Review and oversight requirements

11.3.1 Adverse event definition

A clinical adverse event is any unfavorable or unintended sign, symptom or disease temporally associated with the use of an investigational product occurring in a research patient during treatment or post-treatment follow-up period, regardless of causality

assessment. This includes adverse clinical or laboratory findings, intercurrent illness, or an exacerbation or progression of a disease/condition present at baseline.
An unexpected adverse event is any adverse experience where the specificity or severity of which is not consistent with the current investigator brochure; or, if an investigator brochure is not required or available, the specificity or severity of which is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

11.3.2 Adverse event reporting

Adverse events will be graded using the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 found on the following website:
http://ctep.cancer.gov/reporting/ctc.html

Laboratory test value abnormalities will not be recorded as adverse events unless they are designated as serious, require treatment, or cause premature withdrawal (see Serious Adverse Events definition below).

Specifications to the grading of acute and chronic GVHD are given in Appendix V and VI.

Causality will be rated as definitely, probably, possibly, or unlikely related, or unrelated to the CD34+ CliniMACS® collection. The Principal Investigator is responsible for making an assessment of whether or not it is reasonable to suspect a causal relationship between the adverse event and the investigational product.

11.3.3 Serious adverse events definition

A serious adverse experience (SAE) is any adverse experience occurring at in a study patient that results in any of the following outcomes:

1. Death
2. Life-threatening (places the subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred)
3. Inpatient hospitalization or prolongs existing hospitalization
4. Persistent or significant disability/incapacity (a substantial disruption of a person's ability to conduct normal life functions)
5. Birth defect/congenital anomaly
6. Any important medical event that may not result in prior listed outcomes but, based upon appropriate medical judgment, may jeopardize the subject, and may require medical and surgical intervention to prevent one of the prior listed outcomes.

11.3.4 Special AE documentation guidelines for this protocol

The following AEs will be recorded in the UCSF Helen Diller Family Comprehensive Cancer Center CTMS database:

1. Any evidence of grade III/IV acute or extensive chronic GVHD using protocol appendices V and VI

2. Toxicities of any organ Grade 3, 4, 5 using CTCAE version 4.0
3. Hospitalization
4. Relapse
5. Any change in conditioning regimen outside of planned
6. Any problems with CliniMACS device during cell separation or inability to achieve CD34+ cell and CD3+ cell target doses.
7. Failure to engraft or graft rejection

11.3.5 Adverse event reporting procedures

Adverse Events (AEs) will be recorded on the OnCore eResearch database, all grade 3-5 expected and unexpected AEs will be recorded and updated at each visit.

Serious Adverse Event Reporting

Serious Adverse Event reporting will be in accordance with the UCSF- Committee on Human Research Regulations and Code of Federal Regulation Title 21 Volume 5 Part 312.32.

UCSF CHR website for guidance in reporting serious adverse events
http://www.research.ucsf.edu/chr/Guide/Adverse_Events_Guidelines.pdf

FDA website for guidance in reporting serious adverse events
http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr+312.32

MedWatch forms and information:
http://www.fda/gov/medwatch/getforms.html

CliniMACS® CD34+ Reagent System:
Fax MedWatch form directly to the Safety Officer, Miltenyi Biotec Inc. (781-782-1920)

Serious Adverse events will be reported on the MedWatch form when it meets the definition of expedited FDA reporting.

If the SAE is death and determined to be possibly, probably, or definitely related to the investigational product or any research related procedure, the event must be reported via phone or in person to the UCSF DSMC chair or his designee within 24 hours of the Principal Investigator's awareness of the event. Written documentation of the verbal communication will be sent via e-mail to the DSMC Chair or his designee by the reporting investigator; a copy of the e-mail must be sent to the DSMC Administrator and DSMC Coordinator.

All subjects/patients with serious adverse experiences must be followed up for outcome.

11.3.6 Review of adverse event rates

If the study has an increase of unexpected or expected Adverse Events grade 3 or 4 above the rate reported in the protocol or investigational brochure or device documentation, this will be reported to the DSMC at the time of identifying the increased rate, and each quarterly report will indicate if the AE incidence is within the expected range. If at any time the Investigator stops enrollment or stops the study due to safety issues, the DSMC Chair and Administrator must be notified within 24 business hours via email. The DSMC must receive a formal letter within 10 business days and the CHR must be notified.

12.0 ETHICAL ASPECTS

12.1 Regulatory considerations

This study will be reviewed by the UCSF Comprehensive Cancer Center Protocol Review Committee, in addition to the UCSF Committee on Human Research (IRB, see below). In addition, the study protocol will be filed with the FDA as a new study under BB-IDE#8817, in addition to current study CC#01151. As per FDA regulations, (21 CFR 812.150), annual reports will be submitted to the FDA within 60 days of the anniversary date that the IDE went into effect.

12.2 Institutional Review Board

This protocol and the informed consent will be approved by the UCSF CHR (IRB). The Principal Investigator is responsible for keeping the CHR advised of the progress of the study and of any changes made in the protocol prior to implementation. The Principal Investigator will also keep the CHR informed of any significant adverse reactions, and any protocol exceptions or deviations. Records of all study review and approval documents must be kept on file by the Principal Investigator and are subject to FDA inspection during or after completion of the study. The CHR will receive notification of the termination of the study.

13.0 DATA FORMS AND SUBMISSION SCHEDULE

Forms will be completed and documented for each patient. A research chart with completed paper case report forms and supporting documentation of reportable AEs and SAEs for all enrolled patients will be maintained in a locked cabinet in the BMT office. Patient enrollment information and toxicity/reporting information will also be entered by the coordinator into the CTMS database.

FORMS	COMPLETION SCHEDULE
Enrollment forms: • Recipient Eligibility Checklist • Donor Eligibility Checklist • Recipient Baseline Information	After the subject and donor have given informed consent and have been screened, and prior to recipient beginning conditioning therapy or donor receiving G-CSF prior to apheresis.

• Signed Consents	
Treatment forms: • Transplant Data Form • Cell Processing Report	Completed transplant form one week after conditioning treatment. Within one week of the donor cell processing complete processing form.
Follow-up forms: • Acute GVHD Form • Post-transplant Assessment Form	Starting at 4 weeks after transplant, following protocol-specified time points (see Appendix IV)
AE/SAE reporting: • Study AE/SAE Form • CHR AE/SAE reporting • FDA Med Watch 3500a	See section 11.3 for reportable AEs and SAEs

APPENDICES

APPENDIX I – Alternative conditioning regimens:

Chemotherapy Alone Regimen (for patients who have a contraindication to TBI or non-malignant disease (22).

1. On day –7, administer melphalan 140mg/m2 IV over 30 minutes x 1 dose.

2. On day –6, thiotepa 5mg/kg/dose will be administered intravenously in two doses (each dose of 4 hours) for a total dose of 10mg/kg/day.

3. On days –6 through –2, fludarabine 40 mg/m2/d (infuse over 30 minutes) will be administered in the early morning hours on each of the five days (there will be at least 24 hrs between the last dose of fludarabine and the stem cell infusion). Fludarabine dose will be reduced by 20% for creatinine clearance of < 70 ml/min/1.73m2.

4. Rabbit ATG (thymoglobulin) 2.5 mg/kg on days –3 to day –1 infused over 6 hours.

Reduced Intensity Regimen (for patients with decreased organ function) (31,32)

1. On day -7 through day -3, fludarabine 30 mg/m2/d (infused over 30 minutes) will be administered in the early morning hours on each of the five days. Fludarabine dose will be reduced by 20% for creatinine clearance of 30-70 ml/min/1.73m2.

2. On day –3, thiotepa 5mg/kg/dose will be administered intravenously in two doses (each dose of 4 hours) for a total dose of 10mg/kg/day.

3. On days –2 and –1, melphalan 60 mg/m2/d (infuse over 30 minutes) will be administered in the morning (there will be at least 24 hrs between the last dose of melphalan and the stem cell infusion).

4. Rabbit ATG (thymoglobulin) 0.5 mg/kg on day -7 infused over 4 hrs and then 2.5 mg/kg for four daily doses from day –6 to day –3 infused over 6 hours.

Regimen for chromosome breakage disorders like Fanconi anemia with aplasia (34)

1. On day -7, 300 cGy TBI with thymic shielding per protocol.

2. On day -6 to day -3, fludarabine 35 mg/m^2/day IV over 1 hour. Fludarabine dose will be reduced for decreased creatinine clear and and for children under 1 year of age per protocol.

3. Day -6 to day -3, cyclophosphamide 10mg/kg/day over 2 hours with mesna administration per protocol.

4. On days -6 to -2, equine ATG 30mg/kg/day IV over 4 hours.

Regimen for chromosome breakage disorders like Fanconi anemia with MDS/AML (35)

1. On day -7, a 450 cGy single dose of total body irradiation is delivered.

2. On day -5 through day -2, cyclophosphamide 10mg/kg/dose will be administered intravenously (each dose to run over 1 hour) on each of the four days.

3. On day -6 through day -2, fludarabine 30 mg/m2/d (infused over 30 minutes) will be administered in the early morning hours on each of the five days (there will be at least 24 hrs between the last dose of fludarabine and the stem cell infusion). Fludarabine dose will be reduced by 20% for creatinine clearance of < 70 ml/min/1.73m2.

4. Rabbit ATG (thymoglobulin) 0.5 mg/kg on day -6 infused over 4 hrs and then 2.5 mg/kg for four daily doses from day -5 to day -2 infused over 6 hours

Appendix II – DONOR (pre-administration through collection)

Assessments/Tests	w/in 30 days	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
History & Physical	X						
Serological Testing[1]	X						
ABO Typing	X						
LFTs, CBC/diff, platelet count, creatinine, CMP, Mg, Ca	X						
ECG	X						
CXR	X						
HLA Typing	X						
Pregnancy test[2]	X						
G-CSF[3]		X	X	X	X		X[4]
Apheresis						X	X[4]
CD34+ Selection							X

[1] Serological testing for CMV,syphilis, HIV including p24, EBV, HSV, Hepatitis B C (must be done or repeated within 30 days of donation), HTLV I/II.

[2] If female of child-bearing age.

[3] The donor will receive 4 days of G-CSF (Neupogen) administered subcutaneously (8 g/kg/dose) twice a day. On day 5 donor will undergo apheresis.

[4] Additional days (Day 5, 6, 7) of G-CSF may be given if the donor does not mobilize enough stem cells. Then apheresis will occur on Day 8.

APPENDIX III – RECIPIENT (pre-administration through transplant)

Time Period →	Pre-administration		Cytoreductive Regimen									
	w/in 30 days	w/in 2 weeks	Day -9	Day -8	Day -7	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Transplant (Day 0)
PFTs	X											
ECHO & ECG	X											
Creatinine clearance	X											
Infectious disease testing[1]	X											
ABO typing	X											
Anti-HLA Antibodies	X											
LFTs	X											
Creatinine	X											
Marrow/LP[2]		X										
HLA Typing	X											
History & Physical	X											
CXR or CT (chest)	X											
Pregnancy test[3]	X											
TBI			X	X	X							
Fludarabine4						X	X	X	X	X		
Thiotepa						X						
rATG (Thymoglobulin®)[4]									X	X	X	
Transplant												X

[1]Serological Testing for CMV, HIV (including p24), EBV, HSV, Hepatitis B, C, HTLV I/II.
[2]Within 2 weeks of admission for patients with leukemia/MDS.
[3]If female of childbearing age.
[4]Alternative conditioning regimen may be used based on disease diagnosis as stated in Appendix I

APPENDIX IV – RECIPIENT (follow-up schedule)

	Weeks Post-BMT							Months Post-BMT									
Time Point	**0**	**1**	**2**	**3**	**4**	**8**	**12**	**6 [A]**	**9 [A]**	**12 [B]**	**15 [A]**	**18 [A]**	**21 [A]**	**24**	**36**	**48**	**60**
Engraftment					X	X	X	X	X	X	X	X	X	X	X	X	X
Lymphocyte Phenotyping					X	X	X	X	X	X	X	X	X	X	X	X	X
Mitogen Studies[C]							X	X	X	X	X	X	X	X	X	X	X
Coombs[D]							X	X	X	X							
Kidney function							X	X	X	X		X		X			
Liver function							X	X	X	X		X		X			
PFTs										X				X	X	X	X
ECHO										X							

[A] Recommended follow-up timepoints per standard post-transplant care

[B] Patients will be followed for outcomes related to the device including acute and chronic GVHD and engraftment at study-specified timepoints through Day +100 (Week 12). Subsequent follow-up care is standard for all transplant patients, and data will be collected for OS, DFS, and other secondary endpoints through 2 years post-transplant to fulfill study objectives, and up to 5 years if standard follow-up data are available.

[C] Direct and indirect Coombs test will be checked q 2 weeks beginning at 8 weeks post BMT until the CD4>200 and PHA≥30% of the lower limit of normal control because of the risk of autoimmune hemolytic anemia.

[D] Mitogen studies can be discontinued once results are normal at physician discretion.

APPENDIX V – Acute GVHD staging and grading for children**

ORGAN	STAGE	DESCRIPTION
SKIN	1	Maculopapular rash < 25% of BSA
	2	25 – 50% of BSA
	3	Generalized erythroderma
	4	Desquamation and bullae
LIVER	1	Bilirubin 2 – 3 mg/dL
	2	Bilirubin 3.1 – 6 mg/dL
	3	Bilirubin 6.1 – 15 mg/dL
	4	Bilirubin > 15 mg/dL
GUT	1	Diarrhea > 500 – 1000 ml/day (> 280 mL/m^2 – 556 mL/m^2/day) OR persistent UGI symptoms
	2	Diarrhea > 1000 – 1500 ml/day (> 556 mL/m^2 – 833 mL/m^2/day)
	3	Diarrhea > 1500 ml/day (> 833 mL/m^2/day)
	4	Severe abdominal pain or ileus

GRADE	Skin		Liver		Gut
I	1-2		0		0
II	3	and/or	1	and/or	1
III	--		2-3	and/or	2-3
IV	4	and/or	4	and/or	4

** Adapted from Przepiorka and Jacobsohn articles (41, 42).

APPENDIX VI - Chronic GVHD staging and grading for children**

LIMITED CHRONIC GVHD

1. Localized skin involvement and/or
2. Hepatic dysfunction due to chronic GVHD

EXTENSIVE CHRONIC GVHD

Either:

1. Generalized skin involvement, or

2. Localized skin involvement and/or hepatic dysfunction due to chronic GVHD

   Plus:

   a) Liver histology showing chronic aggressive hepatitis, bridging necrosis, or cirrhosis, or

   b) eye involvement (Schirmer test with < 5mm wetting), or

   c) involvement of minor salivary glands or oral mucosa demonstrated on labial biopsy, or

   d) involvement of any other target organ

3. Involvement of two target organs

** Adapted from Glucksberg (43).

APPENDIX VII - Device information

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. When using the CD34 Reagent, the system selects CD34+ cells from heterogeneous hematological

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

University of California, San Francisco CliniMACS® cell sorter Device information:
1) Serial number: 000288
2) Catalogue number: 15101
3) Part number: 44085

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

APPENDIX VIII - SOP *#206.04 Recipient Selection and Treatment Regimen for Pediatric Bone Marrow Transplant (PBMT).*

---Attached (7 pages)---

APPENDIX IX - SOP *#206.04 Donor and Donor Source Selection for Pediatric Bone Marrow Transplant (PBMT).*

---Attached (11 pages)---

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