CLINICAL RESEARCH PROTOCOL

Title: Safety and efficacy of Miltenyi CliniMACS® CD34 Reagent System for transplant protocol utilizing haploidentical CD34+ selected cells combined with single unit umbilical cord blood transplant for treatment of high-risk hematologic disorders.

Version: February 22, 2021
COMIRB #: 16-1672

Principal Investigator: Jonathan Gutman, MD

IDE #: *17240*

IDE Holder: Jonathan Gutman, M.D.

Product: Miltenyi CliniMACS® CD34 Reagent System

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

Estimated duration of study: 8 years

Enrollment:

	Number	Sex	Age Range
Subjects	Up to 200	Either	≥ 18 - ≤ 80

Table of Contents

STATEMENT OF COMPLIANCE 4
1. SUMMARY 5
2. OBJECTIVE 6
3. BACKGROUND 6
3.1. Review 6
4. SCIENTIFIC AND CLINICAL JUSTIFICATION 7
5. STUDY DESIGN 8
6. ELIGIBILITY ASSESSMENT 9
6.1. Inclusion Criteria - Recipient 9
6.2. Exclusion Criteria – Recipient (any of the following) 9
6.3. Donor Eligibility 9
7. CLINICAL EVALUATION OF THE TRANSPLANT RECIPIENT 10
8. TREATMENT PLAN 10
8.1. Preparative Regimens 10
8.1.1. Preparative Regimen for patients with hematologic malignancies 10
8.1.2. Medium intensity preparative regimen for patients with hematologic malignancies 10
8.1.3. Reduced intensity conditioning (RIC) preparative regimen for patients with hematologic malignancies who cannot tolerate high intensity regimen 11
8.1.4. Preparative Regimen with high dose total body irradiation (TBI) for patients with hematologic malignancies 11
8.1.5. Preparative Regimen for patients with aplastic anemia 11
8.2. Stem Cell Transplantation (Day 0) 12
8.2.1. Cord blood: 12
8.2.2. Haploidentical donor: 12
8.3. GVHD Prophylaxis: 12
9. BIOSTATISTICAL CONSIDERATIONS 13
9.1. Methods of Statistical Analysis 13
9.2. Sample Size 13
9.3. Stopping Rules for Safety 13
10. DATA AND SAFETY MONITORING 15
10.1. Safety Monitoring and Oversight 15
11. REPORTING 17

11.1. Treatment Related Serious Adverse Events 17
11.2. Reports at the time of continuing IRB review 17
11.3. Reporting to Miltenyi Biotec, Inc. 17
11.4. Reporting to the FDA 17
11.4.1. Expectant Outcomes 18
11.5. Data Management 19
11.6. Protocol Monitoring - Quality Control and Quality Assurance 20
12. HUMAN SUBJECT PROTECTIONS 21
12.1. Rationale for Subject Selection 21
12.2. Recruitment 21
12.3. Participation of Children 21
12.4. Hazards and Discomforts 21
12.5. Informed Consent Processes and Procedures 21
13. CliniMACS® CD34 Reagent System *(see current Investigator's Brochure)* 22
13.1. Investigational Product 22
13.2. Physical, Chemical and Toxicological Information 23
13.3. Safety Testing of CliniMACS® System Components 24
13.4. Overall Safety of the CliniMACS® CD34 Reagent System 24
REFERENCES 28

STATEMENT OF COMPLIANCE

This is an investigator-initiated study. The principal investigator (PI), Jonathan Gutman, MD, is conducting the study. University of Colorado is acting as the sponsor.

The trial will be carried out in accordance with Good Clinical Practice (GCP) as required by applicable United States (US) laws and applications, including but not limited to United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 812).

The PI will assure that no changes to the protocol will take place without documented approval from the Institutional Review Board (IRB). All personnel involved in the conduct of this study have completed Human Subjects Protection Training.

I agree to ensure that all staff members involved in the conduct of this study are informed about their obligations in meeting the above commitments.

Principal Investigator (Sponsor Investigator): Jonathan Gutman, MD
Print/Type Name

Signed: _____ **Date:** _____

1. SUMMARY

Allogeneic hematopoietic stem cell transplantation (aHSCT) from an HLA-matched related donor (MRD) can cure patients with hematologic disorders. Unfortunately, many patients with these disorders lack a MRD. For such patients, transplantation using unrelated umbilical cord blood (UCB) has been utilized as an alternative donor transplant strategy. The advantages of UCB transplant (UCBT) include the ease and rapidity of availability, requirement of less than perfect HLA match, and lower rates of graft versus host disease compared to bone marrow or peripheral blood stem cell transplants. The major disadvantage of UCB transplantation in adults is the limited number of nucleated cells contained within the cord unit resulting in prolonged neutropenia which contributes to infection and transplant related mortality (TRM). In order to harness the advantage of UCB availability and to overcome the disadvantage of delayed neutrophil recovery, co-administration of unrelated UCB and highly purified haploidentical peripheral blood CD34+ cells from a related donor promotes rapid engraftment and reduce TRM secondary to prolonged neutropenia associated with conventional UCBT 1-12.

Miltenyi Biotec's CliniMACS® CD34 Reagent System was approved as a Humanitarian Use Device (HUD) by the U.S. FDA. The approved label indication is "The CliniMACS® CD34 Reagent System is indicated for processing hematopoietic progenitor cells collected by apheresis (HPC, 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 graft versus host disease (GVHD) prophylaxis in patients with acute myeloid leukemia (AML) in first morphologic complete remission". While sufficient clinical data exists to warrant the use of haploidentical CD34+ selected cells combined with single unit umbilical cord blood as a standard of care stem cell transplant strategy, the strategy requires CD34+ cells selection using the Miltenyi CliniMACS® CD34 Reagent System. Though the Miltenyi CliniMACS® CD34 Reagent System is used for this purpose at numerous centers[1-12], the device is not FDA approved for this specific indication. As such this investigational device exemption (IDE) protocol supports the use of Miltenyi CliniMACS® CD34 Reagent System for preparing CD34+ enriched/T-cell depleted cells from haploidentical mobilized peripheral blood.

The haploidentical donor will be mobilized by G-CSF and undergo apheresis to collect CD34+ stem cell product after Miltenyi CD34+ selection. The products will be cryopreserved until the time of transplantation. Recipients with hematologic malignancies will receive a standard conditioning. After the conditioning regimen, subjects will receive an allograft on day 0 containing donor CD34+ cells that have been positively selected and T-cell depleted following G-CSF mobilization (goal CD34+ cell dose of 3 x 10^6 CD34+ cells /kg recipient) combined with a single UCB unit (serologically matched at $\geq$ 4/6 HLA loci). UCB unit will not be manipulated, and will be prepared and infused separately following standard of care procedure.

This study will evaluate 200 patients with hematologic disorders meeting indication for transplant but who do not have MRD. Safety will be monitored continuously with a stopping rule for toxicity based on the treatment-related serious adverse event rate (TRSAE), defined by failure of Miltenyi CliniMACS® CD34 Reagent System to select CD34+ cells, and/or failure to engraft that is higher than historical control (<10% for hematologic malignancies and 34% for severe aplastic anemia).

2. OBJECTIVE

To determine safety and efficacy of Miltenyi CliniMACS® CD34 Reagent System to select haploidentical CD34+ cells.

3. BACKGROUND

3.1. Review

Haploidentical donor stem cell transplantation, umbilical cord blood transplant, and Haploidentical CD34+ selected cells combined with single unit umbilical cord blood transplant:

Although allogeneic stem cell transplantation (allo-SCT) is a curative option for many hematologic disorders, MRD are available for only 30% of the patients needing a transplant. Alternative stem cell source includes cord blood or a partially matched (haploidentical) family donor. Potential donors for haploidentical stem cell transplantation (haplo-SCT) include family members (parents, siblings, or children) who share one haplotype with the recipient. Haplo-SCT may have advantages over UCBT for the following reasons: 1) higher stem cell dose and rapid hematological recovery, 2) feasibility of repeated cell collections for post-transplant immunotherapy[13,14]. The major disadvantage of haploidentical transplant is high rate of acute and chronic GVHD without significant immunosuppression, or high rates of relapse if significant immunosuppression is administered.

UCB is also increasingly being used as a source of stem cells, as this allows the transplantation of patients without a MRD. Advantages of UCBT include: 1) the rapidity by which UCB units can be obtained, 2) low relapse rates, 3) the requirement for less than a perfect HLA match, and 4) lower rates of graft versus host disease associated with HLA mismatching compared to bone marrow or peripheral blood stem cell transplants. The major disadvantage of UCBT in adults is the limited number of nucleated cells contained within the cord unit resulting in prolonged neutropenia and failure of engraftment, which contributes to infection and TRM. Median time to neutrophil engraftment is prolonged at day 22 (range, 21-25 days), with less than 10% graft failure for patients with hematologic malignancies. Median

time to neutrophil engraftment for patients with severe aplastic anemia is even further prolonged at 42 days (range, 13 to 55 days), with the primary graft failure incidence of 34%[15-18].

Engraftment is defined as achievement of absolute neutrophil count (ANC) of ≥500 cells/µl for three consecutive days.

Primary graft failure for cord blood transplant (also known as failure to engraft), is defined as failure to achieve a neutrophil count (ANC) of ≥500/µl by day 42 post-transplant and the aplastic appearance of a marrow sample.

Delayed (secondary) graft failure is defined by failure to achieve a neutrophil count (ANC) of ≥500/µl after initial achievement of ANC recovery to >500/µL for three consecutive occasions.

In order to harness the advantage of UCB availability and to overcome the disadvantage of delayed neutrophil recovery, co-administration of unrelated umbilical cord blood and highly purified haploidentical peripheral blood CD34+ cells from a related donor promotes rapid engraftment and reduce TRM secondary to prolonged neutropenia associated with conventional UCBT [1-12].

4. SCIENTIFIC AND CLINICAL JUSTIFICATION

Miltenyi Biotec's CliniMACS® CD34 Reagent System was approved as a Humanitarian Use Device (HUD) by the U.S. FDA. The approved label indication is "The CliniMACS® CD34 Reagent System is indicated for processing hematopoietic progenitor cells collected by apheresis (HPC, 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 graft versus host disease (GVHD) prophylaxis in patients with acute myeloid leukemia (AML) in first morphologic complete remission." In this clinical protocol, the CliniMACS® CD34 Reagent System will be used for processing hematopoietic progenitor cells collected by apheresis (HPC, Apheresis) from an allogeneic, HLA-haploidentical, related donor to obtain a CD34+ cell-enriched population for hematopoietic reconstitution following a preparative regimen in patients with hematologic disorders who require transplant. The procedure used to enrich the CD34+ cells will be identical to the procedure used under the HUD.

While sufficient clinical data exists to warrant the use haploidentical CD34+ selected cells combined with single unit umbilical cord blood as a standard of care stem cell transplant strategy, the strategy requires T-cell depletion and enrichment for CD34+ cells using the Miltenyi CliniMACS® CD34 Reagent System1-12. Miltenyi CliniMACS® CD34 Reagent System is used for haploidentical CD34+ selected cells combined with single unit umbilical cord blood transplant at numerous centers, but the device is not FDA approved for this specific indication. This protocol supports

the use of this device under the proposed IDE. We therefore propose this clinical protocol of umbilical cord blood and haploidentical CD34+ cells transplant following standard conditioning regimen as treatment for subjects with hematologic disorders.

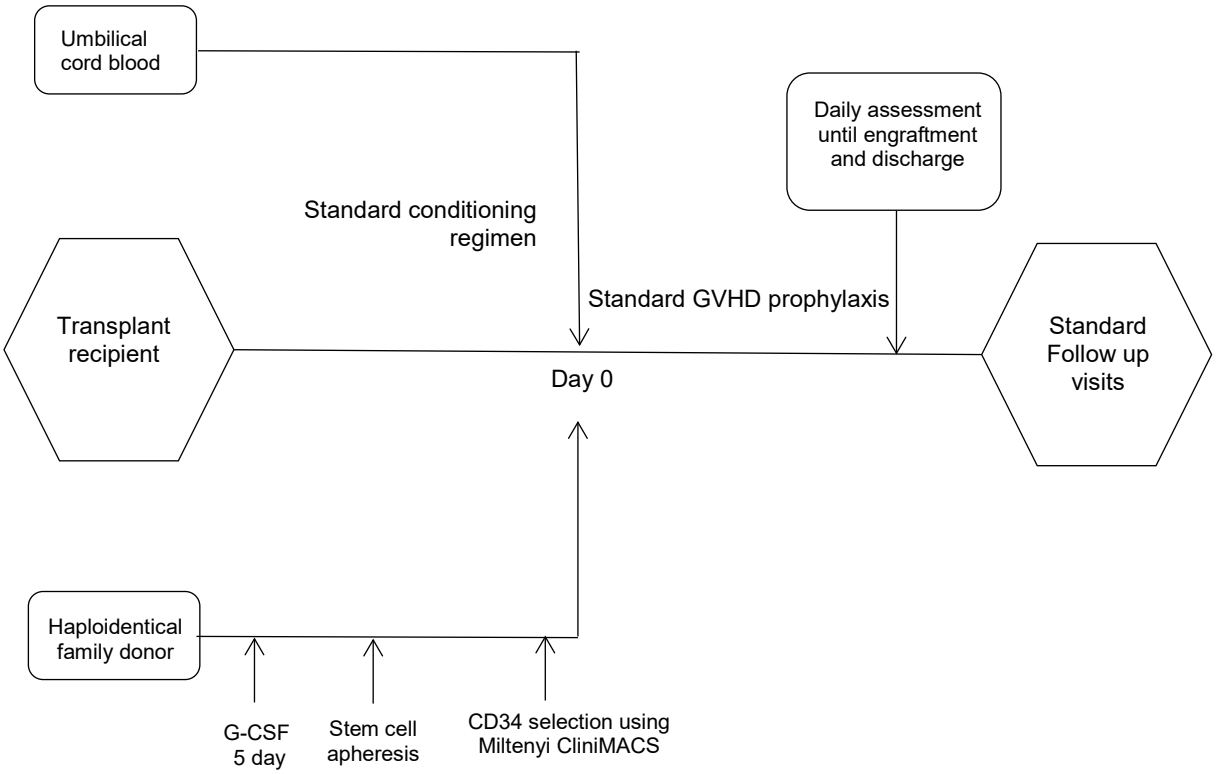
5. STUDY DESIGN

This is an investigator initiated clinical study to evaluate the safety and efficacy of Miltenyi CliniMACS® CD34 Reagent System to promote engraftment.

The haploidentical donor will be mobilized by G-CSF and undergo apheresis to collect CD34+ selected stem cell product after Miltenyi CD34+ selection. The products will be cryopreserved until the time of transplantation.

Recipients will receive a standard conditioning regimen (Please refer to section 8 for dosing and schedule). After the conditioning regimen, the subjects will receive an allograft on day 0 containing donor CD34+ cells that have been positively selected and T-cell depleted following G-CSF mobilization (goal CD34+ cell dose of 3 x 10^6 CD34+ cells /kg recipient) combined with a single UCB unit (serologically matched at ≥ 4/6 HLA loci). UCB unit will not be manipulated, and will be prepared and infused separately following standard of care procedure.

Figure 1. Study Schema



6. ELIGIBILITY ASSESSMENT

6.1. Inclusion Criteria - Recipient

- Ages 18-80 years inclusive
- Diagnosed with high risk hematologic disorders warranting stem cell transplant per institutional standard of care
- Lack HLA-identical related donor
- Availability of at least one HLA- haploidentical (i.e. $\geq$ 5/10 and $\leq$ 8/10 HLA match) related donor (HLA-A, B, C, DR, and DQ loci) who is available to donate CD34+ cells.
- Availability of at least one 4/6 HLA-matched (HLA-A, B, and DR loci) cord blood unit from the National Marrow Donor Program (NMDP). The cord blood unit must contain a minimum TNC (prior to thawing) of at least $2x10^7$ cells per kilogram of recipient body weight
- Ability to comprehend the nature of the treatment

6.2. Exclusion Criteria – Recipient (any of the following)

- HLA identical (6/6) related donor available and readily accessible at time of transplantation evaluation
- Any patient not meeting institutional standard guidelines for transplant eligibility

6.3. Donor Eligibility

- Follow standard of care donor eligibility procedure, outlined in the standard operation procedure (SOP).
- Deferral of donors that:
    1) Have traveled to active Zika virus zones (if donor happed to travel to Zika zone, patient will discuss with treating physician and sign medical necessity form).
    2) Are at potential risk of transmissible spongiform encephalopathy (TSE), including Creutzfeldt–Jakob disease (CJD), based on family and travel history

7. CLINICAL EVALUATION OF THE TRANSPLANT RECIPIENT

All clinical care will be provided according to institutional standard practice for stem cell transplants.

8. TREATMENT PLAN

Patients will be treated according to the standard of care treatment plan outlined below.

8.1. Preparative Regimens

8.1.1. Preparative Regimen for patients with hematologic malignancies

- **Fludarabine (Flu):** 30 mg/m^2/dose IV infusion administered once daily for 5 doses on Days -6, -5, -4, -3, -2 (total dose of 150 mg/m^2).
- **Cyclophosphamide (Cy):** 50 mg/kg/dose IV infusion administered once daily for 1 dose on Days -6 (total dose of 50 mg/kg).
- **Thiotepa:** 5mg/kg/dose IV infusion administered once daily for 2 doses on Days -5, -4 (total dose of 10 mg/kg).
- **Total body irradiation (TBI):** Timing and dose: 4 Gy of total body irradiation (TBI) will be administered in 2 fractions over 2 days on days -2 to -1.

8.1.2. Medium intensity preparative regimen for patients with hematologic malignancies

- **Fludarabine (Flu):** 30 mg/m^2/dose IV infusion administered once daily for 5 doses on Days -6, -5, -4, -3, -2 (total dose of 150 mg/m^2).
- **Cyclophosphamide (Cy):** 50 mg/kg/dose IV infusion administered once daily for 1 dose on Days -6 (total dose of 50 mg/kg).
- **Thiotepa:** 5mg/kg/dose IV infusion administered once on day -5 (total dose of 5mg/kg).
- **Total body irradiation (TBI):** Timing and dose: 4Gy of total body irradiation (TBI) will be administered in 2 fractions over 2 days on days -2 to -1.

8.1.3. Reduced intensity conditioning (RIC) preparative regimen for patients with hematologic malignancies who cannot tolerate high intensity regimen

- **Fludarabine (Flu):** 40 mg/m^2/dose IV infusion administered once daily for 5 doses on Days -6, -5, -4, -3, -2 (total dose of 200 mg/m^2).
- **Cyclophosphamide (Cy):** 50 mg/kg/dose IV infusion administered once daily for 1 dose on Days -6 (total dose of 50 mg/kg).
- **Total body irradiation (TBI):** Timing and dose: 3 Gy of total body irradiation (TBI) will be administered on day -1.

8.1.4. Preparative Regimen with high dose total body irradiation (TBI) for patients with hematologic malignancies

- **Fludarabine (Flu):** 25 mg/m^2/dose IV infusion administered once daily for 3 doses on Days -7, -6, -5 (total dose of 75 mg/m^2).
- **Cyclophosphamide (Cy):** 60 mg/kg/dose IV infusion administered for 2 doses on Days -6, -5 (total dose of 120 mg/kg).
- **Total body irradiation (TBI):** Timing and dose: 12Gy of total body irradiation (TBI) will be administered twice a day over 3 days on days -3 to -1.

8.1.5. Preparative Regimen for patients with aplastic anemia

- **Cyclophosphamide (Cy):** 60 mg/kg/dose IV infusion administered once daily for 2 dose on Days -7, -6 (total dose of 120 mg/kg).
- **Fludarabine (Flu):** 25 mg/m^2/dose IV infusion administered once daily for 5 doses on Days -5, -4, -3, -2, -1 (total dose of 125 mg/m^2).
- **Atgam 40mg/kg/dose** for 4 doses on Days -5, -4 , -3, -2 (total dose of 160 mg/kg).
- **Total body irradiation (TBI):** Timing and dose: 4 Gy of total body irradiation (TBI) will be administered over 1 day on Day -1.

8.2. Stem Cell Transplantation (Day 0)

8.2.1. Cord blood:

Unit will be selected and prepared following institutional standard guidelines: Minimum total nucleated cells (TNC) (prior to thawing) of 2 x 10^7 per kilogram of recipient body weight. Confirmatory typing reveals a minimum antigen level match in 4/6 HLA loci. Shipment of the cord blood unit will not be requested until the G-CSF mobilized T-cell depleted graft collected from the haploidentical donor contains a suitable number of CD34+ cells (≥2 x 10^6/kg) and CD3+ T-cells (<1 x 10^4 CD3+ cells/ kg).

8.2.2. Haploidentical donor:

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

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

8.3. GVHD Prophylaxis:

Cyclosporine: Cyclosporine will be initiated at a dose of 2.5 mg/kg IV BID starting on day -3. The cyclosporine dose will be adjusted to obtain goal therapeutic drug levels of 200-400 ng/mL. When the recipient is able to take oral medications, cyclosporine will be give PO in two divided doses every 12 hours, and will be continued for at least 6 months.

Mycophenolate mofetil (MMF): 1 gram administered orally or intravenously three daily starting day 0. When the recipient is able to take oral medications, MMF will be given PO in two divided doses every 12 hours, and will be continued until around day 30 and tapered off.

9. BIOSTATISTICAL CONSIDERATIONS

Primary Endpoint: To determine the rate of successful engraftment of patients who received haploidentical CD34+ selected cells combined with single unit umbilical cord blood transplant. Failure defined as failure of Miltenyi CliniMACS® CD34 Reagent System to select CD34+ cells, and/or failure to engraft by day 42.

9.1. Methods of Statistical Analysis

There will be 200 individuals who may be included in this study of safety analysis. The primary outcome of this study is the proportion of individuals who have had a successful engraftment by the end of the 42 day period. Assuming a sample size of 200, if there is an engraftment success rate of 75% (or a failure rate of 25%), there would be a resulting 95% confidence interval of 63% to 87% (that is a width of 12% on either side of 75% successful engraftments).

9.2. Sample Size

A maximum of 200 subjects will be evaluated for safety analysis.

9.3. Stopping Rules for Safety

Safety will be monitored continuously with a stopping rule for toxicity based on the treatment-related serious adverse event rate (TRSAE). Safety monitoring will continue until engraftment.

Stopping rule for TRSAE is defined as failure of Miltenyi CliniMACS® CD34 Reagent System to select CD34+ cells, and/or failure to engraft. The number of TRSAE which will end the study are based on an acceptable rate of 10% of patients with hematologic malignancies and 20% of patients with severe aplastic anemia having adverse events.

Table 1: Stopping rules for patients with hematologic malignancies.

Number of subjects in the experiment	Stop if the number of subjects who develop any TRSAE reaches
≤5	3
≤10	4
≤15	5
≤20	6
≤26	7
≤32	8
≤39	9
≤46	10

The termination conditions which yield a probability of termination of 5% when the rate of adverse events is at 10%.

Table 2: Stopping rules for patients with severe aplastic anemia.

Number of subjects in the experiment	Stop if the number of subjects who develop any TRSAE reaches
≤3	3
≤4	4
≤8	5
≤11	6
≤14	7
≤17	8
≤20	9
≤23	10
≤27	11
≤30	12
≤34	13
≤38	14
≤41	15
≤45	16
≤49	19

The termination conditions which yield a probability of termination of 5% when the rate of adverse events is at 20%.

10. DATA AND SAFETY MONITORING

10.1. Safety Monitoring and Oversight

The sponsor investigator will be responsible for monitoring the trial per the trial monitoring plan, in addition to overseeing the safety and efficacy of the trial including any specimens collected, executing the data and safety monitoring (DSM) plan, and complying with all reporting requirements to local and federal authorities. This oversight will be accomplished through additional oversight from the Data and Safety Monitoring Committee (DSMC) at the University of Colorado Cancer Center (CU Cancer Center). The DSMC is responsible for ensuring data quality and study participant safety for all clinical studies at the CU Cancer Center, which is the coordinating institution of this trial. A summary of the DSMC's activities is as follows:

- Conduct of internal audits
- Ongoing review of all unanticipated adverse device effects, serious adverse events (SAEs) and unanticipated problems (UAPs)
- May submit recommendations for corrective actions to the CU Cancer Center's Executive Committee

Per the CU Cancer Center Institutional DSM Plan, SAEs and UAPs are reported to the DSMC, IRB and the sponsor investigator per protocol. All SAEs and UAPs including unanticipated adverse device effects are to be reported to the DSMC within 7 (for fatal or life-threatening events) or 15 (non-life-threatening events) calendar days of the sponsor investigator receiving notification of the occurrence.

Each subject's treatment outcomes will be discussed by the site PI and appropriate staff at weekly meetings. Data regarding number of subjects, adverse device effects, treatment modifications and treatment responses will be discussed and documented in the meeting's minutes.

The sponsor investigator will provide a DSM progress report to the CU Cancer Center DSMC on a recurring basis (either every six or twelve months based on DSMC vote). The DSM report will include a protocol summary, current enrollment numbers, summary of adverse device effects to include

specific unanticipated adverse device effects, SAEs, UAPs and AEs, any treatment modifications, all protocol deviations, and protocol amendments. The DSM report submitted to the DSMC will also include, if applicable, the results of any efficacy data analysis conducted. Results and recommendations from the review of this progress report by the DSMC will then be provided to the sponsor investigator in a DSMC review letter. The sponsor investigator is then responsible for ensuring this letter is submitted to the site's IRB of record at the time of IRB continuing review.

Site monitoring visits will be performed by the sponsor investigator's authorized representative on a regular basis, pursuant to the Monitoring Plan. During these visits, information recorded on the CRFs will be verified against source documents. Additional computer programs that identify selected protocol deviations, out-of-range data, and other data errors within the electronic data entry may also be used to help monitor the study. As necessary, requests for data clarification or correction will be sent to the appropriate site PI. Independent auditors from the sponsor investigator's authorized representative will be allowed by the site's PI to audit. In addition, audits may be conducted at any time by appropriate regulatory authorities and/or the IRB.

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

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

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

U.S. Food and Drug Administration
Center for Biologics Evaluation and Research
Document Control Center

10903 New Hampshire Avenue
WO71, G112
Silver Spring, MD 20993-0002

In compliance with 21 CFR 812.150(a), the principal investigator will submit the following reports: unanticipated adverse device effects, withdrawal of IRB approval, progress report, deviations from the investigational plan, if the investigator uses a device without obtaining informed consent, and a final report. 21 CFR 812.150(a) specifies time frames for these reports.

11. REPORTING

11.1. Treatment Related Serious Adverse Events

TRSAE is defined as failure of Miltenyi CliniMACS® CD34 Reagent System to select CD34+ cells, and/or failure to engraft.

Reports to the IRB: The PI must report TRSAE as soon as possible but not more than 5 days after the PI first learns of the event.

For device, the PI must report to the IRB any deviation from the investigational plan to protect the life or physical well-being of a subject in an emergency as soon as possible, but no later than 5 working days.

11.2. Reports at the time of continuing IRB review

At continuing review, the PI will provide to the IRB a summary of: All TRSAE

11.3. Reporting to Miltenyi Biotec, Inc.

Reports of any TRSAE observed during the clinical trial and for which there is a relationship with the use of the Miltenyi CliniMACS CD34 Selection System and/or its components will also be forwarded as soon as possible to Miltenyi Biotec.

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

11.4. Reporting to the FDA

IDE Unanticipated Adverse Device Effects Report (Refer to 21 CFR 812.50): The IDE sponsor, Jonathan Gutman, MD, will report suspected

unexpected serious adverse reaction (SUSARs), and any unanticipated adverse device effect observed during the clinical trial for which there is a relationship with the use of the Miltenyi CliniMACS CD34 Selection System and/or its components on the conduct of the study to the FDA as soon as possible, but in no event later than 10 working days after the sponsor learns of the effect. Thereafter, the sponsor shall submit such additional reports concerning the effects as FDA requests.

Any positive sterility test result will be reported to FDA within 30 calendar days after initial receipt of the positive culture test results.

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

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

11.4.1. Expectant Outcomes

The following are expected events or outcomes for the any type of transplant recipient and will be documented in the subject's medical record. However, only TRSAE as defined in Section 11.1 will be reported for the purpose of this study.

- Renal insufficiency
- Hepatic insufficiency
- Transient cardiac arrhythmias
- Transient cardiac insufficiency
- Pulmonary insufficiency
- Neutropenia and its complications
- Thrombocytopenia and its complications
- Anemia and its complications
- Transfusion reactions

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

Should any of the above events result in death while a subject is being followed for engraftment, it will be documented in the electronic Case Report Form (eCRF) and study record as the subject not completing the study or reaching engraftment, if applicable. The diagnosis at death will also be captured in the study record and eCRF.

11.5. Data Management

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

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

***End of study procedures**:* Data will be stored in restricted access area and in a password protected database until it is no longer of scientific value. Identifiable data will not be sent outside University of Colorado without prior IRB approval or appropriate conditions for disclosure outlined in the executed MTA or CTA.

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

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

11.6. Protocol Monitoring - Quality Control and Quality Assurance

Site monitoring visits will be performed by the sponsor investigator's authorized representative on a regular basis, pursuant to the Monitoring Plan. During these visits, information recorded on the CRFs will be verified against source documents. Additional computer programs that identify selected protocol deviations, out-of-range data, and other data errors within the electronic data entry may also be used to help monitor the study. As necessary, requests for data clarification or correction will be sent to the appropriate site PI.

Independent auditors from the sponsor investigator's authorized representative will be allowed by the site's PI to audit. In addition, audits may be conducted at any time by appropriate regulatory authorities and/or the IRB.

12. HUMAN SUBJECT PROTECTIONS

Patients will be treated according to the standard of care treatment plan.

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

12.1. Rationale for Subject Selection

This protocol is open to males and females from all ethnic and racial groups.

12.2. Recruitment

The study will be listed on clinicaltrials.gov, clinical center research studies.

12.3. Participation of Children

Patients less than 18 years of age will not be eligible in this study.

12.4. Hazards and Discomforts

Related to the CliniMACS CD34 Reagent Systems:

Theoretical risks to the patient could include system failure, operator error, or patient reaction to selected product components (i.e. residual amounts of unbound CliniMACS CD 34 Reagent, Iron dextran, or human anti-mouse antibodies). Specifically, theoretical risks are 1) failure of Miltenyi CliniMACS® CD34 Reagent System to select CD34+ cells, and/or 2) failure to engraft. For full text see Investigator's brochure for Miltenyi CliniMACS CD34+ system, Version 7, dated 6/13/2011, section 6, Summary and Guidance for the Investigator).

Transplant procedure will be carried out as a standard of care per institution guidelines.

12.5. Informed Consent Processes and Procedures

Miltenyi CliniMACS® CD34 Reagent System will be explained to the patient. The research objectives of this trial, the procedure and its attendant risks and discomforts will be carefully explained to the subject and a signed informed consent document will be obtained prior to entry onto this study. The PI and delegated personnel will lead this discussion.

13. CliniMACS® CD34 Reagent System *(see current Investigator's Brochure)*

13.1. Investigational Product

Miltenyi Biotec's CliniMACS® CD34 Reagent System was approved as a Humanitarian Use Device (HUD) by the U.S. FDA. The approved label indication is "The CliniMACS® CD34 Reagent System is indicated for processing hematopoietic progenitor cells collected by apheresis (HPC, 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 graft versus host disease (GVHD) prophylaxis in patients with acute myeloid leukemia (AML) in first morphologic complete remission.

In this clinical protocol, the CliniMACS® CD34 Reagent System will be used for processing hematopoietic progenitor cells collected by apheresis (HPC, Apheresis) from an allogeneic, HLA-haploidentical, sibling donor to obtain a CD34+ cell-enriched population for hematopoietic reconstitution following a preparative regimen in patients with severe hematologic disorders who require transplant. The procedure used to collect the cells will be identical to the procedure used under the HUD.

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

13.2. Physical, Chemical and Toxicological Information

- CD34 Reagent Description: the CliniMACS CD34 Reagent is a dark amber, nonviscous, colloidal solution containing the antibody conjugate in buffer. The conjugate consists of a monoclonal antibody towards the class II epitope of the human CD34 antigen. The murine monoclonal IgG1 antibody is covalently linked to dextran beads that have an iron oxide/hydroxide core and are superparamagnetic.
- Safety testing of the CD34 Monoclonal Antibody: cell banking, cell culture, as well as subsequent purification of the antibody, follow the applicable current international guidelines as described in Section 4 of the Investigator's brochure for Miltenyi CliniMACS CD34+ system, Version 7, dated 6/13/2011. The testing of the CD34 Master Cell Bank, the End of Production Cells, the CD34 mAb pooled cell culture harvest (unprocessed bulk) and the purified CD34 monoclonal antibody (mAb) have been completed and the purified CD34 mAb has been released for manufacturing of the CD34 Reagent. Additionally, the viral inactivation/removal steps used in the purification of the CD34 monoclonal antibody have been validated.
- Safety testing of the CD34 Reagent: detailed toxicity studies have been undertaken to assess the safety of the CD34 Reagent when delivered in dosages significantly greater than the projected maximum dosage anticipated in clinical use. The testing was performed in accordance with 21 CFR §58, Good Laboratory Practices for Nonclinical Laboratory Studies. A summary of this testing is provided in **Table 3**.

Table 3: Toxicity studies to assess the safety of the CD34 Reagent

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

13.3. Safety Testing of CliniMACS® System Components

Instrument, Tubing Sets and PBS/EDTA Buffer - Biocompatibility testing of the CliniMACS System components (Tubing Sets and PBS/EDTA Buffer) was performed according to ISO 10993. The requirements of ISO 10993 were fulfilled for the CliniMACS CD34 Reagent System. The CliniMACS plus Instrument has been tested for electrical safety and the potential for fire, shock, explosion, or mechanical damage. Potential safety issues have been reduced by using a design to meet European standards EN 60601-1. It is UL and CSA listed and approved.

13.4. Overall Safety of the CliniMACS® CD34 Reagent System

The results summarized in this Investigator's Brochure support that CliniMACS CD34 Reagent System is sufficiently safe for clinical use with

human subjects. The potential application of the CliniMACS CD34 Reagent is broad. Infusion of purified CD34+ cells is indicated in a number of clinical applications after myeloablative or lymphoablative therapy including reduction of tumor cells in the transplant and depletion of T cells for autologous (autoimmune diseases) and allogeneic transplantations.

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

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

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

Cells were isolated from leukapheresis products from sixty-five subjects enrolled in the study. Fifty-four subjects received selected CD34+ cells and fifty-two were evaluable for engraftment as summarized in the table below (one patient died 5 days post-transplant and prior to engraftment and one patient did not recover platelet counts even after back-up cells were infused). All subjects receiving selected cells completed 60 and 100-day follow-up after infusion,

during which time their hematological and immune status were monitored, as was HAMA production. A summary of the results of the European Clinical Trial is provided below.

Table 4: European Clinical Trial results

Time to Hematological Engraftment After Infusion of CliniMACS Selected CD34+ Cells		
Time to Engraftment (Days)		
	Platelets (≥ 20 x 10^9/L)	Neutrophils (≥ 500/uL)
Median (Kaplan Meier)	11.6	9.1
SD	6.05	5.81
Quartile range (Kaplan Meier)	10.0-12.0	8.0-10.0
Range	8-29	8-11
Number	52	52

The following conclusions were made regarding this clinical study:

- The CliniMACS CD34 Reagent System selects CD34+ cells from heterogeneous hematological cell populations. The resulting CD34+ product is of high purity (median of 96.1 %, range 27.4 – 99.4 %); the median recovery of CD34+ cells was 52.3 % (range 15.2 – 146.3%). The reported performance results are similar to those seen in pre-clinical studies.
- Infusion of selected, autologous CD34+ cells after high dose chemotherapy resulted in rapid engraftment (see Table 4). These data are comparable to previously reported results, using bone marrow or peripheral blood cells as stem cell source. Following cyclophosphamide, thiotepa and carboplatin (CTCb), Weaver et al. reported median times to platelet and neutrophil engraftment of 9 days (range 0-53 days) and 9 days (range 5-26 days), respectively. Also after CTCb, Elias et al., reported median times to platelet and neutrophil recovery of 12 days (range 8-134 days) and 14 days (range 10-57 days), respectively19,20.
- The selection process has no discernable effect on cell viability or

sterility.

- There were no adverse events or device malfunctions reported as related to the infusion of the cells or use of the CliniMACS CD34 Reagent System. None of the subjects were reactive for HAMA post infusion. There were no reports of late engraftment failure or evidence of delayed immune reconstitution.

REFERENCES

1. Nicole J. Gormley JW, Hahn Khuu, Jeremy Pantin, Theresa Donohue, Roger Kurlander, Sawa Ito, Minoo Battiwalla, A. John Barrett, Sophie Grasmeder, Lisa Cook, Catalina Ramos, Patricia Prince, David Stroncek, Willy A. Flegel, Maria Berg, Robert Reger, Charles D. Bolan, Sharon Adams, Richard Childs. Co-Infusion of Allogeneic Cord Blood with Haploidentical CD34+ Cells Improved Transplant Outcome for Patients with Severe Aplastic Anemia Undergoing Cord Blood Transplantation. *Blood* 2011: S118.
2. Xin Tian JW, Nicole Gormley, Hahn Khuu, David Stroncek, Susan F. Leitman, Roger Kurlander, Elena Cho, Lisa Cook, Catalina Ramos, Ladan Foruraghi, Charles D. Bolan, Richard W. Childs. NK Cell KIR Ligand Mismatches Influence Engraftment Following Combined Haploidentical and Umbilical Cord Blood (UCB) Transplantation In Patients With Severe Aplastic Anemia (SAA). *Blood* 2013: 122.
3. Bautista G, Cabrera JR, Regidor C, et al. Cord blood transplants supported by co-infusion of mobilized hematopoietic stem cells from a third-party donor. *Bone marrow transplantation* 2009; **43**(5): 365-73.
4. Kwon M, Bautista G, Balsalobre P, et al. Haplo-cord transplantation using CD34+ cells from a third-party donor to speed engraftment in high-risk patients with hematologic disorders. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2014; **20**(12): 2015-22.
5. Magro E, Regidor C, Cabrera R, et al. Early hematopoietic recovery after single unit unrelated cord blood transplantation in adults supported by co-infusion of mobilized stem cells from a third party donor. *Haematologica* 2006; **91**(5): 640-8.
6. Enkhtsetseg Purev GA, Ritesh Kotecha, Jennifer Wilder, Hahn M. Khuu, David F. Stroncek, Roger Kurlander, Robert N. Reger, Willy A. Flegel, Sharon Adams, Lisa Cook, Catalina Ramos, Elena Cho, Richard W. Childs. Excellent Engraftment and Long-Term Survival in Patients with Severe Aplastic Anemia (SAA) Undergoing Allogeneic Hematopoietic Stem Cell Transplantation (HSCT) with Haplo-Identical CD34+ Cells Combined with a Single Umbilical Cord Blood Unit. *Blood* 2015: 126.
7. Jain N, Liu H, Artz AS, et al. Immune reconstitution after combined haploidentical and umbilical cord blood transplant. *Leuk Lymphoma* 2013; **54**(6): 1242-9.
8. Van Besien K, Liu H, Jain N, Stock W, Artz A. Umbilical cord blood transplantation supported by third-party donor cells: rationale, results, and applications. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation*

2013; **19**(5): 682-91.

9. Juliet N. Barker DMP, Parastoo Dahi,, Sean Devlin KE, Marissa Lubin,, Richard Meagher HC-M, Jenna D. Goldberg AAJ, Guenther Koehne. Double-Unit Cord Blood (CB) Transplantation Combined with Haplo-Identical CD34+ Cells Results in 100% CB Engraftment with Enhanced Myeloid Recovery. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2104; **20**: S128-S50.
10. Liu H, van Besien K. Alternative donor transplantation--"mixing and matching": the role of combined cord blood and haplo-identical donor transplantation (haplo-cord SCT) as a treatment strategy for patients lacking standard donors? *Curr Hematol Malig Rep* 2015; **10**(1): 1-7.
11. Tsai SB, Liu H, Shore T, et al. Frequency and Risk Factors Associated with Cord Graft Failure after Transplant with Single-Unit Umbilical Cord Cells Supplemented by Haploidentical Cells with Reduced-Intensity Conditioning. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2016; **22**(6): 1065-72.
12. van Besien K, Hari P, Zhang MJ, et al. Reduced intensity haplo plus single cord transplant compared to double cord transplant: improved engraftment and graft-versus-host disease-free, relapse-free survival. *Haematologica* 2016; **101**(5): 634-43.
13. Bacigalupo A, Dominietto A, Ghiso A, et al. Unmanipulated haploidentical bone marrow transplantation and post-transplant cyclophosphamide for hematologic malignanices following a myeloablative conditioning: an update. *Bone marrow transplantation* 2015; **50 Suppl 2**: S37-9.
14. Raiola AM, Dominietto A, di Grazia C, et al. Unmanipulated haploidentical transplants compared with other alternative donors and matched sibling grafts. *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2014; **20**(10): 1573-9.
15. Cornetta K, Laughlin M, Carter S, et al. Umbilical cord blood transplantation in adults: results of the prospective Cord Blood Transplantation (COBLT). *Biology of blood and marrow transplantation : journal of the American Society for Blood and Marrow Transplantation* 2005; **11**(2): 149-60.
16. Gutman JA, Ross K, Smith C, et al. Chronic graft versus host disease burden and late transplant complications are lower following adult double cord blood versus matched unrelated donor peripheral blood transplantation. *Bone marrow transplantation* 2016.

17. Barker JN, Fei M, Karanes C, et al. Results of a prospective multicentre myeloablative double-unit cord blood transplantation trial in adult patients with acute leukaemia and myelodysplasia. *British journal of haematology* 2015; **168**(3): 405-12.
18. Eapen M, Klein JP, Ruggeri A, et al. Impact of allele-level HLA matching on outcomes after myeloablative single unit umbilical cord blood transplantation for hematologic malignancy. *Blood* 2014; **123**(1): 133-40.
19. 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; **79**(11): 3036-44.
20. Weaver CH, Hazelton B, Birch R, et al. An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. *Blood* 1995; **86**(10): 3961-9.