CliniMACS® CD34 Reagent System

Rx only

For *in vitro* use only.

HUMANITARIAN DEVICE:

Authorized by U.S. Federal law for use in the treatment of patients with acute myeloid leukemia (AML) in first complete remission. The effectiveness of the device for this use has not been demonstrated.

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INDICATIONS FOR USE

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.

CONTRAINDICATIONS

Do not use $CD34^+$ cells prepared with CliniMACS® CD34 Reagent System in patients with known hypersensitivity to murine (mouse) proteins or iron-dextran.

DESCRIPTION

The CliniMACS® CD34 Reagent System is a medical device system that consists of the following components:

- CliniMACS® CD34 Reagent - dark amber, non-viscous, colloidal solution containing an antibody conjugate in buffer. The conjugate consists of a murine IgG_1 monoclonal antibody directed against the Class II epitope of the human CD34 antigen, which is chemically conjugated to dextran beads having an iron oxide/hydroxide core. (See the CliniMACS® CD34 Reagent Package Insert for more information.)
- CliniMACS® plus Instrument - software-controlled instrument that processes the HPC, Apheresis. (See the CliniMACS® User Manual for the CD34 Reagent System for more information.)
- CliniMACS® Tubing Set TS or Tubing Set LS - a single-use, sterile, disposable tubing set with two proprietary cell separation columns. The CliniMACS® Tubing Set TS is for processing HPC, Apheresis preparations containing up to 0.6×10^9 $CD34^+$ cells out of a total cell number not exceeding 60×10^9 white blood cells. The CliniMACS® Tubing Set LS is for larger scale preparations containing up to 1.2×10^9 $CD34^+$ cells out of a total cell number not exceeding 120×10^9 white blood cells. (See the CliniMACS® Tubing Sets Package Insert and the CliniMACS® User Manual for the CD34 Reagent System for more information.)
- 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* processing of HPC, Apheresis. (See the CliniMACS® PBS/EDTA Buffer Package Insert and the CliniMACS® User Manual for the CD34 Reagent System for more information.)

PRINCIPLES OF OPERATION

The CliniMACS® CD34 Reagent System is an *in vitro* medical device system used to select and enrich $CD34^+$ cells from HPC, Apheresis while passively depleting other cells, such as $CD3^+$ T cells, which cause graft versus host disease. The system is based on "magnetically-activated cell sorting" (MACS) employing antibodies conjugated to iron-containing particles that can be attracted to a magnetic field (referred to as "magnetic labeling"). Using the specificity of anti-CD34 antibody interaction with cell surface CD34 antigen found on hematopoietic progenitor cells, the system enriches $CD34^+$ cells from HPC, Apheresis by passing the antibody-labeled cell suspension through a separation column with a strong magnetic gradient. The separation column retains the magnetically labeled $CD34^+$ target cells while unlabeled cells flow through and are collected in the Negative Fraction Bag. Several automated washing steps are performed, disposing most of the liquid into the Buffer Waste Bag. The magnetically-selected $CD34^+$ cells are released from the separation column when the magnet is disengaged, removing the magnetic field, and the target $CD34^+$ cells are eluted into the Cell Collection Bag.

WARNINGS

- Do not infuse the CliniMACS® CD34 Reagent or the CliniMACS® PBS/EDTA Buffer into patients directly.
- Hypersensitivity Reactions

  Hypersensitivity reactions, including anaphylaxis, have been observed during infusion of $CD34^+$ cells from the CliniMACS® CD34 Reagent System. Monitor the patient for hypersensitivity reactions, including anaphylaxis, during infusion of $CD34^+$ cells from the CliniMACS® CD34 Reagent System.
- Engraftment failure

  Failure to infuse an adequate number of functioning $CD34^+$ cells can result in engraftment failure. Collect sufficient HPC, Apheresis to yield at least 2.4×10^6 $CD34^+$ cells per kg of patient body weight after system processing (see Device Performance below). The clinical trial (see Clinical Performance below) using the CliniMACS® CD34 Reagent System to process HPC, Apheresis did not test allografts with less than 2.4×10^6 $CD34^+$ cells per kg of recipient body weight. Monitor patients for laboratory evidence of hematopoietic recovery after transplantation.
- Acute and chronic graft versus host disease (GVHD)

  GVHD can occur in patients who receive HPC, Apheresis processed using the CliniMACS® CD34 Reagent System. Use pharmacologic prophylaxis if more than 1×10^5 $CD3^+$ cells per kilogram of recipient body weight are infused.
- Delayed immune reconstitution after transplantation

  Removal of T cells from the HPC, Apheresis can delay immune reconstitution after transplantation. Patients who receive the $CD34^+$ cell-enriched population prepared using the CliniMACS® CD34 Reagent System are at risk for serious opportunistic viral infections, including post-transplant lymphoproliferative disorder caused by Epstein-Barr virus (EBV) and cytomegalovirus (CMV). Monitor for EBV and CMV in the peripheral blood of patients after transplantation and initiate appropriate treatment promptly.

PRECAUTIONS

- Safety and probable benefit in children under the age of 17 years have not been established.
- Drugs may be incompatible with the CliniMACS® PBS/EDTA Buffer. Do not add drugs to the buffer other than Human Serum Albumin as specified in the CliniMACS® User Manual for the CD34 Reagent System.
- Do not use cryopreserved and thawed HPC, Apheresis because cryopreservation promotes cell clumping, which may lead to device performance issues. Process HPC, Apheresis as soon as available, but not longer than 24 hours after collection.
- Use only HPC, Apheresis from an allogeneic, HLA-identical sibling donor with the CliniMACS® CD34 Reagent System.
- Collect HPC, Apheresis according to standard hospital or institutional leukapheresis procedures in standard leukapheresis collection bags. Do not include additional anticoagulants or blood additives, such as heparin, other than those normally used during leukapheresis. Keep the HPC, Apheresis at controlled room temperature (+19 °C to +25 °C (67° to 77° F)) if it has to be stored, e.g., overnight, before processing. Do not allow the concentration of leukocytes to exceed 0.2×10^9 cells per mL.
- Only trained operators should use the CliniMACS® CD34 Reagent System to prepare $CD34^+$ cells for infusion. Operator training is provided by Miltenyi Biotec authorized personnel.

PROCEDURES

Refer to the CliniMACS® User Manual for the CD34 Reagent System for complete instructions. An on-line version is available at www.cd34-aml.com/usermanual.

Do not connect the CliniMACS® CD34 Reagent System to the patient at any time.

Collect the following data on the leukapheresis product before starting the preparation of the HPC, Apheresis for enrichment:

- Total number of leukocytes
- Percentage of $CD34^+$ cells
- Total number of $CD34^+$ cells
- Percentage of $CD3^+$ cells
- Total number of $CD3^+$ cells
- Viability

The $CD34^+$ cells are enriched in the following four steps:

Step 1 - Immunomagnetic Labeling of $CD34^+$ Hematopoietic Progenitor Cells

Add the CliniMACS® CD34 Reagent to the HPC, Apheresis and allow the reagent to bind to the $CD34^+$ cells. After incubation, remove unbound reagent from the suspension. The cells are now ready for selection in an automated continuous flow selection process using the CliniMACS® plus Instrument.

NOTE: Exceeding the capacity for either total cell number or $CD34^+$ cell number may impact the performance of the device. The standard-scale capacity for the enrichment of $CD34^+$ cells using the CliniMACS® CD34 Reagent System with one vial of CD34 Reagent and the CliniMACS® Tubing Set TS is 0.6×10^9 $CD34^+$ cells out of a total cell number not exceeding 60×10^9 cells. Large-scale capacity for the enrichment of up to 1.2×10^9 $CD34^+$ cells out of a total cell number of 120×10^9 cells (large-scale application) requires two vials of the CliniMACS® CD34 Reagent and the CliniMACS® Tubing Set LS.

Step 2 - Choice of Program

Choose CD34 Selection 1/2 program on the CliniMACS® plus Instrument.

Step 3 - Installation of Tubing Set

- Install tubing set (TS or LS).
- Attach Cell Collection Bag to the tubing set.
- Follow the prompts provided on the CliniMACS® plus Instrument screen to complete the tubing set installation.
- Join the bag containing the cells from Step 1 to the sterile CliniMACS® Tubing Set.

Step 4 - Selection of CD34+ Cells

Process the cells through the CliniMACS® plus Instrument using the CD34 Selection program. The instrument selects cells by passing the immunomagnetically-labeled suspension through the separation column, in which strong magnetic gradients are generated. The separation column retains the immunomagnetically-labeled $CD34^+$ target cells while unlabeled cells flow through and are collected in the Negative Fraction Bag. Automated washing steps dispose of excess liquid into the Buffer Waste Bag. The retained $CD34^+$ cells are released from the separation column when the magnet is disengaged, and the target $CD34^+$ cells are eluted into the Cell Collection Bag.

Establish the suitability of the target $CD34^+$ cells before infusion. Examine the following parameters:

- Total number of leukocytes
- Total viability
- Total number of $CD34^+$ cells
- Total number $CD3^+$ cells
- Purity and recovery of $CD34^+$ cells
- CD3 log depletion

See Table 1 for performance values observed in the clinical trial.

Assessment of the non-target fraction for the total number and viability of leukocytes is recommended to assess the performance of the device and quality of the device output ($CD34^+$ cells).

DO NOT infuse the $CD34^+$ cells in CliniMACS® PBS/EDTA Buffer! Exchange the CliniMACS® PBS/EDTA Buffer contained in the $CD34^+$ target fraction to a clinical grade infusion solution appropriate for infusion into humans prior to infusion of the $CD34^+$ cells.

See the CliniMACS® User Manual for the CD34 Reagent System for full instructions. The CliniMACS® User Manual for the CD34 Reagent System includes instructions on the preparation of solutions and samples, as well as a detailed list of equipment and materials that are required for $CD34^+$ cell selection. (See Chapter 3 of the CliniMACS® User Manual for the CD34 Reagent System.)

PERFORMANCE CHARACTERISTICS

Adverse Reactions

The safety of the CliniMACS® CD34 Reagent System was evaluated in a clinical trial that included 44 subjects with AML undergoing HPC, Apheresis transplantation from an HLA-identical sibling donor.[1] There were 16 males and 28 females of median age 49 years (range, 21 to 60 years). The myeloablative preparative regimen included total body irradiation, thiotepa, cyclophosphamide, and rabbit antithymocyte globulin. The median number of $CD34^+$ cells infused was 7.9×10^6 per kg (range 2.4 to 30.4). The median number of $CD3^+$ cells infused was 0.07×10^5 per kg (range 0.01 to 0.83).

Among the 44 subjects, there were no grades 3 to 5 infusion reactions, no allergic reactions, and no graft failures. Testing for development of human anti-mouse antibodies (HAMA) was not performed. A severe or life-threatening infection was reported for 38% of the subjects. An infection by any virus was reported for 61% of the subjects, and the 1-year incidence of EBV infection in particular was 25%. One subject (2%) developed a fatal post-transplantation lymphoproliferative disorder.

Device Performance

The safety and feasibility of use of the CliniMACS® CD34 Reagent System was evaluated in a multicenter, single-arm, clinical trial. In this study, allogeneic donors were mobilized with daily subcutaneous granulocyte colony-stimulating factor (G-CSF) at a dose of 10 to 16 µg per kg per day. Leukapheresis was performed on a continuous flow cell separator commencing on Day 5 of G-CSF treatment, and $CD34^+$ cell enrichment of the HPC, Apheresis was performed using the CliniMACS® CD34 Reagent System. Most donors underwent at least two, but not more than three, apheresis to reach the post-selection enrichment target of greater than 5.0×10^6 $CD34^+$ cells per kg recipient body weight while maintaining less than 1.0×10^5 $CD3^+$ cells per kg recipient body weight.

Eighty-four selection procedures were performed on HPC, Apheresis collected from a total of 44 donors. The minimum number of $CD34^+$ cells required for transplantation, greater than 2×10^6 per kg recipient body weight, was achieved for 100% (44) of donors. This was attained with one apheresis for 93% (41) of the donors and two apheresis for an additional 7% (3). The target number of $CD34^+$ cells, greater than 5×10^6 per kg recipient body weight, was achieved for 84% (37) of the 44 donors. This target number was attained with one apheresis for 36% (16), with two apheresis for 45% (20), and with three apheresis for 2% (1) of the 44 donors. Device performance is shown in the table below.

Table 1: Device Performance Summary; N=84

Attributes Measured		Mean	Std Dev	Median	Min	Max
Starting TNC $\times 10^{10}$		7.46	3.26	6.95	2.1	18.0
Initial Viability (%)		97.60	2.74	99.0	86.9	100.0
$CD34^+$ Cells $\times 10^7$	Starting Count	59.71	41.09	47.85	7.3	208.0
	Final Count	36.90	25.05	29.80	6.1	119.0
Final $CD34^+$ Yield (%)		66.06	20.25	65.00	29.9	125.6
Final $CD34^+$ Purity (%)		93.03	8.31	96.65	61.5	99.8
$CD3^+$ T Cells $\times 10^8$	Starting Count	179.45	69.80	168.50	55.00	362.00
	Final Count	0.00652	0.01039	0.00217	0.00026	0.04971
Log_{10} $CD3^+$ T-Cell Depletion		4.78	0.55	4.90	3.2	5.9
Final Viability (%)		96.57	3.84	97.70	74.0	100.0
Total $CD34^+$ Cells Infused/kg $\times 10^6$		8.81	5.21	7.924	2.41	30.360
Total $CD3^+$ Cells Infused/kg $\times 10^6$		0.015	0.020	0.0066	0.0011	0.08328

Clinical Performance

The clinical trial included 37 subjects with AML in first complete remission (CR) undergoing transplantation. All donors were HLA-identical siblings. The study subjects included 14 (37.8%) males and 23 (62.2%) females of median age 48 years (range: 21 to 60 years). The cytogenetics risk group was intermediate for 68%, unfavorable for 27%, and unknown for 5% of subjects. The myeloablative preparative regimen included total body irradiation, thiotepa, cyclophosphamide, and rabbit antithymocyte globulin. The median number of $CD34^+$ cells infused was 7.4×10^6 per kg recipient body weight (range: 2.4 to 30.4). The median number of $CD3^+$ cells infused was 0.07×10^5 per kg recipient body weight (range: 0.01 to 0.63). No immunosuppressive drugs were administered for prevention of GVHD.

All subjects achieved an absolute neutrophil count that exceeded 0.5×10^9 per liter by Day 21 after transplantation. The platelet count recovered to greater than 20×10^9 per liter by Day 100 for 91.9% (95% CI, 82.4 to 100%). There was one late graft failure. At Day 100 after transplantation, the cumulative incidence of grades 2 to 4 acute GVHD was 27% (95% CI, 14 to 42%), and that for grades 3 to 4 acute GVHD was 5% (95% CI, 1 to 16%). The cumulative incidence of chronic GVHD at 2 years after transplantation was 19% (95% CI, 8 to 33%).

Additional Safety Assessment

The potential risks of using the CliniMACS® CD34 Reagent System were evaluated via a Data Analysis Protocol (DAP), which retrospectively compared the Day-100, 1-year, and 2-year endpoints in the clinical trial to the same endpoints as had been measured in a historical control group of patients that had used a conventional pharmacologic method of GVHD prophylaxis. Table 2 shows the results of the comparison.

Table 2: Comparison of the Single-Arm CliniMACS® CD34 Reagent System Study to historical controls using pharmacological immunosuppression

Endpoints	Single-Arm CliniMACS® CD34 Reagent System (n=37) % (95% CI)	Historical Controls Using pharmacological immunosuppression (n=65) % (95% CI)
% Neutrophil Engraftment at Day 30 (>500/µL)*	100	100+
% Platelet Engraftment at Day 30 (>20,000/µL)*	92 (82.4, 100)	84#(72.5, 91.4)
Acute GVHD at Day 100, Grades 2–4*	27 (13.9, 42.0)	35 (23.9, 47.0)
Acute GVHD at Day 100, Grades 3–4*	5 (1, 16.1)	9 (3.7, 17.8)
Chronic GVHD at 2 years*	19 (8.2, 33.0)	49 (36.5, 61.0)
Relapse Rate at 2 years*	16 (6.5, 29.9)	28 (17.7, 39.7)
Non-relapse Mortality at 2 years*	20 (8.5, 34.5)	14 (6.8, 23.4)
Overall Survival at 2 years	67 (48.8, 79.7)	67 (54.1, 77.2)
Disease-free Survival at 2 years	64 (46.0, 77.4)	58 (44.8, 68.9)
GVHD-free Survival at 2 years	46 (29.6, 60.9)	18 (9.6, 28.2)

* Cumulative Incidence
\+ neutrophil engraftment data missing for two patients
\# Platelet data missing for one patient

REFERENCES

1 Devine SM, Carter S, Soiffer RJ, Pasquini MC, Hari PN, Stein A, Lazarus HM, Linker C, Stadtmauer EA, Alyea EP 3rd, Keever-Taylor CA, O'Reilly RJ. Low Risk of Chronic Graft-versus-Host Disease and Relapse Associated with T Cell-Depleted Peripheral Blood Stem Cell Transplantation for Acute Myelogenous Leukemia in First Remission: Results of the Blood and Marrow Transplant Clinical Trials Network Protocol 0303. Biology of Blood and Marrow Transplantation 2011;17:1343-1351.