INDICATIONS FOR USE
The CliniMACS® CD34 Reagent System is indicated for processing hematopoietic progenitor cells collected byapheresis (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 consisting 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, 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® ³⁺ Instrument - software-controlled instrument that processes the HPC, Apheresis. (See the CliniMACS® User Manual for the CD34 Reagent System for more information.)
- CliniMACS® Tubing Sets TS or Tubing Set LS - 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 × 10⁹ CD34⁺ cells out of a total cell number not exceeding 60 × 10⁹ white blood cells. The CliniMACS® Tubing Set LS is for larger scale preparations containing up to 1.2 × 10¹⁰ CD34⁺ cells out of a total cell number not exceeding 120 × 10⁹ 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.)
- CliniMACS® CD34 Reagent System

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 antigens 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
- Hyperreactivity 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 × 10⁹ 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 × 10⁹ CD34⁺ cells per kg of recipient body weight. Monitor patients for 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 × 10¹⁰ 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 institute 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. Proceed 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 × 10⁹ 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.cliniacs-amr.com/usermanual. Do not connect the CliniMACS® CD34 Reagent System to the collection bag during processing.

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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 (T5 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 displace 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 CD33+ 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.

The myeloablative preparative regimen included total body irradiation, thiopeta, cyclophosphamide, and rabbit antithymocyte globulin. The median number of CD34+ cells infused was 7.9 × 10^6 per kg recipient body weight, which exceeded 0.5 × 10^9 per liter by Day 21 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%).

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% (64) 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>
<thead>
<tr>
<th>Attributes Measured</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
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</thead>
<tbody>
<tr>
<td>Starting TNC × 10^10</td>
<td>7.46</td>
<td>3.26</td>
<td>6.95</td>
<td>2.1</td>
<td>18.0</td>
</tr>
<tr>
<td>Initial Viability (%)</td>
<td>97.60</td>
<td>2.74</td>
<td>99.0</td>
<td>86.9</td>
<td>100.0</td>
</tr>
<tr>
<td>CD34+ Cells × 10^7</td>
<td>197.45</td>
<td>69.80</td>
<td>168.50</td>
<td>55.00</td>
<td>362.00</td>
</tr>
<tr>
<td>Total CD3+ Cells</td>
<td>185.80</td>
<td>69.80</td>
<td>168.50</td>
<td>55.00</td>
<td>362.00</td>
</tr>
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<td>168.50</td>
<td>55.00</td>
<td>362.00</td>
</tr>
<tr>
<td>Final CD34+ Yield (%)</td>
<td>66.06</td>
<td>20.25</td>
<td>65.00</td>
<td>29.9</td>
<td>125.6</td>
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<tr>
<td>Final CD34+ Purity (%)</td>
<td>93.03</td>
<td>8.31</td>
<td>96.65</td>
<td>61.5</td>
<td>99.8</td>
</tr>
<tr>
<td>CD3+ T-Cell Count</td>
<td>179.45</td>
<td>69.80</td>
<td>168.50</td>
<td>55.00</td>
<td>362.00</td>
</tr>
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<td>55.00</td>
<td>362.00</td>
</tr>
<tr>
<td>Log10 CD3+ T-Cell Depletion</td>
<td>4.78</td>
<td>0.55</td>
<td>4.90</td>
<td>3.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Log10 CD34+ T-Cell Depletion</td>
<td>4.78</td>
<td>0.55</td>
<td>4.90</td>
<td>3.2</td>
<td>5.9</td>
</tr>
<tr>
<td>Final Viability (%)</td>
<td>96.57</td>
<td>3.84</td>
<td>97.76</td>
<td>74.0</td>
<td>100.0</td>
</tr>
<tr>
<td>Total CD34+ Cells</td>
<td>179.45</td>
<td>69.80</td>
<td>168.50</td>
<td>55.00</td>
<td>362.00</td>
</tr>
<tr>
<td>Infused/kg × 10^6</td>
<td>8.81</td>
<td>5.21</td>
<td>7.04</td>
<td>2.41</td>
<td>30.36</td>
</tr>
<tr>
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<td>179.45</td>
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<td>2.41</td>
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</tr>
</tbody>
</table>

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 22%, and unknown for 5% of subjects. The myeloablative preparative regimen included total body irradiation, thiopeta, 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 × 10^6 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 × 10^6 per liter by Day 21 after transplantation. The platelet count recovered to greater than 20 × 10^6 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

<table>
<thead>
<tr>
<th>Endpoints</th>
<th>Single-Arm CliniMACS® CD34 Reagent System (n=15)</th>
<th>Historical Controls Using immunosuppression (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Neutrophil Engraftment at Day 30 (&lt;1000µL/L)</td>
<td>100*</td>
<td>100*</td>
</tr>
<tr>
<td>% Platelet Engraftment at Day 30 (20,000µL/L)</td>
<td>92 (82.4, 100)</td>
<td>84 (72.5, 91.4)</td>
</tr>
<tr>
<td>Acute GVHD at Day 100, Grades 2–4*</td>
<td>27 (13.9, 42.0)</td>
<td>35 (23.9, 47.0)</td>
</tr>
<tr>
<td>Acute GVHD at Day 100, Grades 3–4</td>
<td>5 (1.6, 16.1)</td>
<td>9 (3.7, 17.8)</td>
</tr>
<tr>
<td>Chronic GVHD at 2 years*</td>
<td>19 (8.2, 33.0)</td>
<td>49 (36.5, 61.0)</td>
</tr>
<tr>
<td>Relapse Rate at 2 years*</td>
<td>16 (6.5, 29.8)</td>
<td>28 (17.7, 39.7)</td>
</tr>
<tr>
<td>Non-relapse Mortality at 2 years</td>
<td>20 (8.5, 34.5)</td>
<td>14 (6.8, 23.4)</td>
</tr>
<tr>
<td>Overall Survival at 2 years</td>
<td>67 (48.8, 79.7)</td>
<td>67 (54.1, 77.2)</td>
</tr>
<tr>
<td>Disease-free Survival at 2 years</td>
<td>64 (46.0, 77.4)</td>
<td>58 (44.8, 66.9)</td>
</tr>
<tr>
<td>GVHD-Free Survival at 2 years</td>
<td>46 (26.0, 60.9)</td>
<td>18 (9.6, 28.2)</td>
</tr>
</tbody>
</table>

* Cumulative Incidence
* Cumulative incidence data missing for two patients
* Platelet data missing for one patient

REFERENCES