DEPARTMENT OF HEALTH AND HUMAN SERVICES

DEC 06 1996

Monica Krieger, Ph.D.
CellPro, Incorporated
22215 26th Avenue SE
Bothell, Washington 98021

Re: BP-94-0001
Product: CEPRATE® SC Stem Cell Concentration System
Filed: January 3, 1994
Amended: June 6, 1994, August 2, 1995, January 15, 1996

Dear Dr. Krieger:

The Center for Biologics Evaluation and Research (CBER) of the Food and Drug Administration (FDA) has completed its review of your premarket approval application (PMA) for the CEPRATE® SC Stem Cell Concentration System. This device is indicated for the processing of autologous bone marrow to obtain a CD34+ cell enriched population which is intended for hematopoietic support after myeloablative chemotherapy. We are pleased to inform you that the PMA is approved subject to the conditions described below and in the "Conditions of Approval" (enclosed). You may begin commercial distribution of the device upon receipt of this letter.

The sale, distribution, and use of this device are restricted to prescription use in accordance with 21 CFR 801.109 within the meaning of section 520(e) of the Federal Food, Drug, and Cosmetic Act (the act) under the authority of section 515(d)(1)(B)(ii) of the act. FDA has also determined that to ensure the safe and effective use of the device that the device is further restricted within the meaning of section 520(e) under the authority of section 515(d)(1)(B)(ii), (1) insofar as the labeling specify the requirements that apply to the training of practitioners who may use the device as approved in this order and (2) insofar as the sale, distribution, and use must not violate sections 502(q) and (r) of the act.

The expiration dating period for the anti-human CD34 biotinylated monoclonal antibody (murine) has been established and approved at 18 months when stored at -70°C. The expiration dating period for all other components of the CEPRATE SC Disposables Kit has been established and approved at 12 months when said components are stored at the appropriate temperatures as designated below. The Avidin column, the Precolumn, and the liter of sterile, non-pyrogenic RPMI 1640 Cell Culture Medium must be stored at 2 - 8°C. The Kit also includes one Tubing Set, one 40 micron Pall® SQ40S Blood Filter, and three liters of sterile, non-pyrogenic phosphate buffered saline which are to be stored at 15 - 30°C.

CBER will publish a notice of its decision to approve your PMA in the FEDERAL REGISTER. The notice will state that a summary of the safety and effectiveness data upon which the approval is based is available to the public upon request. Within 30 days of publication of the notice of approval in the FEDERAL REGISTER, any interested person may seek review of this decision by requesting an opportunity for administrative review, either through a hearing or review by an independent advisory committee, under section 515(g) of the Federal Food, Drug, and Cosmetic Act (the act).

Failure to comply with the conditions of approval invalidates this approval order. Commercial distribution of a device that is not in compliance with these conditions is a violation of the act.

You are reminded that as soon as possible, and before commercial distribution of your device, that you must submit an amendment to this PMA submission with copies of all approved labeling in final printed form.

All required documents should be submitted in triplicate, unless otherwise specified, to the address below and should reference the above PMA number to facilitate processing.

Document Control Center (HFM-585)
Center for Biologics Evaluation and Research
Food and Drug Administration
1401 Rockville Pike
Rockville, Maryland 20852-1448

If you have any questions concerning this approval order, please contact Keith Webber, Ph.D. at (301) 594-5660.

Sincerely yours,

Jay P. Siegel, M.D., FACP
Director
Office of Therapeutics
Research and Review
Center for Biologics
Evaluation and Research

Enclosure

CEPRATE® SC STEM CELL CONCENTRATION SYSTEM

DESCRIPTION

The CEPRATE® SC Stem Cell Concentration System consists of an instrument and a single-use, sterile, prepackaged kit containing disposable components. The CEPRATE® SC Disposable Kit consists of the following components:

(1) Avidin Column
(1) Precolumn
(1) Tubing Set
(3) Sterile, Non-pyrogenic Phosphate Buffered Saline (PBS), 1000 mL
(1) Sterile, Non-pyrogenic RPMI 1640, 1000 mL
(1) 40µm Pall SQ40S Blood Filter
(1) Anti-Human CD34 Biotinylated Monoclonal Antibody (murine), 3.0 mL/ vial

PRINCIPLES OF OPERATION

The CEPRATE® SC System concentrates CD34+ cells using a proprietary, continuous-flow immunoadsorption technique. After marrow cell harvest and buffy-coat preparation, the cells are incubated with biotinylated murine anti-CD34 monoclonal antibody which binds selectively to CD34+ cells. After a wash step to remove excess, unbound antibody, marrow cells are processed through the CEPRATE® SC System. The cells flow through a column containing beads coated with avidin. The biotinylated, antibody-labeled CD34+ cells bind to the avidin-coated beads, and unlabeled cells are washed through the column. The CD34+ cells are then eluted by gentle mechanical agitation of the beads. The cell yield, cell purity and ease of use of this method of stem cell selection have been compared with other methods of stem cell selection (1).

INDICATIONS AND USAGE

The CEPRATE® SC System is indicated for the processing of autologous bone marrow to obtain a CD34+ cell enriched population which is intended for hematopoietic support after myeloablative chemotherapy. Infusion of the CD34+ enriched population results in a lower incidence of DMSO infusion-associated complications compared with infusion of unselected bone marrow cells. It is recommended that sufficient bone marrow be harvested to provide at least 1.2×10^6 CD34+ cells per kg of patient body weight after CD34+ cell selection. Infusion of less than 1.2×10^6 CD34+ cells/kg of recipient body weight has been associated with delayed platelet engraftment [See Clinical Experience].

CLINICAL EXPERIENCE

Description of Clinical Studies:

The safety and effectiveness of the CEPRATE® SC System for enrichment of CD34+ cells from autologous bone marrow for hematopoietic reconstitution were evaluated in 29 patients in a single-arm, pilot trial and in 92 transplanted patients in a phase 3, open-label, randomized trial after high-dose chemotherapy. All patients had stages II-IV breast cancer except four of the 29 patients enrolled in the pilot trial had non-Hodgkin's lymphoma.

The Phase 3 study had two primary objectives; to demonstrate a lower incidence of alterations in hemodynamic parameters (i.e., hypertension, bradycardia) occurring within 24 hours of infusion and to demonstrate equivalent neutrophil engraftment by day 20 post transplant.

Results of Clinical Studies:

In the Phase 3 study, the endpoint for efficacy was a significant difference in one of the hemodynamic side effects listed below:

- Maximum observed post infusion increase in diastolic blood pressure
- Maximum observed post infusion increase in systolic blood pressure
- Maximum observed post infusion decrease in heart rate

Ninety-five women were randomized and 92 underwent transplantation; 45 received autologous marrow processed with the CEPRATE® SC System (selected arm) and 47 received buffy coat preparations of autologous marrow (unselected arm). The mean values for the magnitude of change from baseline of three hemodynamic parameters were significantly less in patients in the selected arm compared with results in patients in the unselected arm. (Table1)

Table 1
Mean Changes in Hemodynamic Parameters within 24 hours Following Marrow Infusion (All Infused Patients)

Hemodynamic Endpoint	Treatment Arm		p-value*
	Selected arm (n=45)	Unselected arm (n=47)	
	(mean ± S.D)		
Maximum increase in systolic blood pressure (mmHg)	9 ± 11	22 ± 14	<.001
Maximum increase in diastolic blood pressure (mmHg)	7 ± 7	16 ± 10	<.001
Maximum decrease in heart rate (beats/min)	9 ± 5	22 ± 11	<.001

*One-sided t-test

The percentage of patients achieving neutrophil engraftment by day 20 was 89% in the selected arm and 88% in the unselected arm. The engraftment rate on the selected arm minus that on the unselected arm was 1% with a 95% confidence interval of -11% to 15%. The median number of days from infusion to neutrophil engraftment was 13 days (95% C.I.: 11-14 days) in the selected arm and 11 days (95% C.I.: 10-12 days) in the unselected arm, a difference of 2 days (95% C.I.: -1 to 5 days).

Patients in the study experienced a variety of adverse events commonly associated with marrow infusion. Patients receiving marrow processed with the CEPRATE® SC System had a lower incidence of infusion-related toxicity compared to those receiving unselected marrow.

All adverse events (grades 1-4) and severe/life threatening adverse events (grade 3 and 4) during the first 24 hours after infusion are listed in Table 2 for the 92 infused patients.

Table 2
Percent of Patients with Infusion-Related Adverse Events

Organ System		Selected Arm (n=45)		Unselected Arm (n=47)	
		All events	Severe/life-threatening	All events	Severe/life-threatening
Cardiovascular	Hypertension (systolic)	9%	2%	47%	0%
	Hypertension (diastolic)	4%	0%	6%	0%
	Cardiac rate/rhythm	33%	0%	87%	0%
GI	Cramping	9%	2%	21%	6%
	Nausea	51%	22%	70%	30%
	Diarrhea	36%	2%	38%	4%
Other	Hemoglobinuria	29	0%	89	9%
	Headache	11%	2%	9%	6%

A number of secondary endpoints were measured (up to day 100) to assess engraftment characteristics (See Table 3). No differences were noted between the two study arms, except in platelet recovery. The median number of days from infusion to date of platelet engraftment ($\geq 20{,}000/mm^3$, without transfusions), was 28 days (95% C.I.:23 - 32 days) in the selected arm and 20 days (95% C.I.:18-23 days) in the unselected arm, a difference of 8 days (95% C.I.: 1-11 days).

Table 3
Engraftment Characteristics and Immediate Post-Transplant Course (All Infused Patients)

Endpoint	Treatment Arm		p-value
	Selected Arm (n=45)	Unselected Arm (n=47)	
	median (range)	median (range)	
Days to ANC $\geq 500/mm^3$	13 (9 - 33)	11 (8 - 48)	0.16
Days to platelet $\geq 20{,}000/mm^3$	28 (11 - 68)	20 (8 - 61)	0.04
RBC transfusions (units/patient)	6 (2 - 30)	8 (2 - 32)	0.11
Platelet transfusions (units/patient)	64 (12 - 264)	54 (8 - 570)	0.44
Days of hospitalization	18 (10 - 40)	17 (10 - 50)	0.50
Percentage of patients w/infection	53%	47%	0.68
Percentage of patients w/ bleeding episodes	20%	26%	0.62

Cox proportional hazards regression models and multivariate regression analyses were used to assess the potential relationship between time to platelet engraftment and laboratory variables in the 89 eligible, infused patients. Patients with total nucleated cells (TNC) at harvest of less than 13.8 x 10^8 cells (<25th percentile) and patients with less than 1.2 x 10^6 CD34+ cells/kg prior to cryopreservation (<25th percentile) were at a significantly increased risk of delayed time to platelet engraftment. Analyses of the data from the 29 patients enrolled in the pilot trials who received selected marrow also showed an increased risk of delayed time to platelet engraftment in those patients with <1.2 x 10^6 CD34+ cells/kg at the time of cryopreservation.

Long-Term Follow-up Results:

Four patients in the selected arm were infused with fewer than 0.5 x 10^6 CD34+ cells/kg. Three of the four patients had delayed platelet engraftment (on days 29, 56, and 84); two of these patients also had neutropenia after initial neutrophil engraftment (one was episodic and one required infusion of back-up marrow at day 211 to achieve hematopoietic reconstitution). Five other patients (four in the selected arm and one in the unselected arm) had leukopenia (leukocyte <2000 cells/µl) at 6 or 12 months post-transplantation. Eight patients required one or more platelet transfusions between day 100 and 12 months post-transplantation; five in the selected arm and three in the unselected arm.

There was evidence of immune reconstitution between 6-12 months post-transplant in patients in both study arms. There were no significant differences between the study arms with regard to median neutrophil count, leukocyte count, hemoglobin, or platelet count at 100 days or after two years. There were no significant differences in the number of platelet transfusions, bleeding episodes or infections during the follow-up period (median time = 2 years).

There were no statistically significant differences between the arms in the probability of overall survival or progression-free survival. The median progression-free survival was 40 weeks in the selected arm and 101 weeks in the unselected arm. Kaplan-Meier curves of progression-free survival in each arm converged at 2½ years. The median overall-survival for the selected arm was 109 weeks; the median had not been reached in the unselected arm.

Progenitor Cell Composition of Marrow Preparations Processed with the CEPRATE® SC System

Processing of bone marrow on the CEPRATE® SC System resulted in an enrichment of CD34+ cells and CFU-GM and a loss in absolute numbers of progenitor cells. Results for all patients (both pilot and phase 3 studies) who received selected bone marrow are shown in Table 4. These results are similar to those reported in the literature (2).

Table 4
Composition of Autologous Marrow Before and After Processing using the CEPRATE® SC Stem Cell Concentration System

		Prior to Selection median (range)	After Selection median (range)
ENRICHMENT (relative proportion of progenitor cells)			
Total CD34+ cells/total nucleated cells	n=73	1.3% (0.2-4.0)	75% (16-92)
CFU-GM/10^5 nucleated cells	n=61	28 (4-112)	1694 (177-18,000)
PROGENITOR CELL NUMBER			
CD34+ cells (x10^6)		248 (53-908)	91 (15-288)
CD34+ cells (x 10^6/kg)		4.1 (0.7-15.9)	1.4 (0.4-4.5)
CFU-GM (x 10^5/kg)		10 (0.9-60)	4 (0.4-21)

CONTRAINDICATIONS

The use of the CEPRATE® SC System is contraindicated in patients whose tumors express the CD34 antigen.

WARNINGS AND PRECAUTIONS

WARNINGS

It is recommended that sufficient bone marrow be harvested to provide at least 1.2 x 10^6 CD34+ cells per kg of patient body weight after CD34 selection. When fewer than 1.2 x 10^6 CD34+ cells per kg of patient body weight are infused, infusion was associated with a significantly increased risk of delayed engraftment of platelets in retrospective analyses of both the phase 3 and pilot studies. A prospectively validated threshold, which more precisely identifies the number of progenitor cells required to minimize the risk of delayed engraftment, has not been determined. In addition, absolute measurements of CD34+ cells are known to vary widely; therefore, the CD34 threshold value provided should not be viewed as definitive.

PRECAUTIONS

- Operate and maintain the CEPRATE® SC System according to the Operator Manual. CellPro will not be responsible for decreased System performance if procedures are other than those specified by CellPro. Only trained personnel should operate the CEPRATE® Instrument and prepare cell products for infusion.
- The fluid pathways of the tubing set are sterile and nonpyrogenic. Do not use if package integrity is compromised or moisture is present inside package.
- The CEPRATE® SC Disposable Kit is intended for single use only. DO NOT REUSE COMPONENTS.

  Treat all blood products as though they contain an infectious agent. Follow institutional guidelines regarding the handling of infectious agents. Dispose of all materials used in this procedure as biohazardous waste. Use aseptic technique for all procedures.
- All tubing and electrical cables should be inspected. Ensure that all connections are secure and that tubing is not kinked or bent before beginning the procedure.
- Do not locate the instrument next to equipment that vibrates. Movement or vibration may affect system performance.
- Individuals with pacemakers should consult their physician to determine if it is safe for them to be near the CEPRATE® Instrument. The agitator motor of the instrument contains magnets that may affect pacemaker operation.
- Keep software diskettes away from the CEPRATE® Instrument agitator assembly; the magnets can destroy data on a diskette.
- If liquid spills onto the instrument, immediately wipe and dry the surface of the instrument.
- Electrical installations should comply with applicable local electrical codes and the CellPro operator manual.
- Store cryovials in the vapor phase above liquid nitrogen. Cryovials immersed in liquid nitrogen can develop leaks. Liquid nitrogen present inside the cryovial can shatter the vial during the thawing procedure. Therefore, appropriate safety equipment should be used when thawing cryovials.
- Caution: Federal law restricts the use of this device to trained personnel.

ADVERSE EVENTS

Delayed Engraftment

Infusion of low number of CD34+ selected autologous bone marrow cells has been associated with delayed engraftment (See Warnings).

Marrow Sterility

Bone marrow preparations were cultured in 89 of the 95 patients in the phase 3 trial including cultures before and after processing with the CEPRATE® SC System. In 3 patients, marrow that was culture negative prior to processing became culture positive after selection (2 *Staphylococcus*, coagulase-negative cultures, and one *Aspergillus* species culture).

Human Anti-Mouse Antibody (HAMA) Response

Processing of cells in the CEPRATE® SC Stem Cell Concentration System results in a theoretical possibility of exposing patients to murine protein; this possibility should be taken into consideration prior to infusion of selected cells into individuals with known hypersensitivity to products of murine origin. Sixty patients were evaluated at baseline, day 30, and day 100 for the presence of a human anti-murine antibody response. One of 29 patients in the selected arm and two of 31 patients in the unselected arm had anti-murine antibody titers which were more than two standard deviations above that of a group of normal subjects.

Infusion-related Toxicity

All adverse events (grades 1-4) and severe/life threatening adverse events (grade 3 and 4) during the first 24 hours after infusion are listed in Table 2. Most adverse events occurring within 24 hours of infusion were less common in the selected than in the unselected control arm (see Clinical Experience).

INSTRUCTIONS FOR USE

Materials Provided

The CEPRATE® SC Disposable Kit consists of the following components:

- (1) Avidin Column
- (1) Precolumn
- (1) Tubing Set
- (3) Sterile, Non-pyrogenic Phosphate Buffered Saline (PBS), 1000 mL
- (1) Sterile, Non-pyrogenic RPMI 1640, 1000 mL
- (1) 40µm Pall SQ40S Blood Filter
- (1) Anti-CD34 Biotinylated Monoclonal Antibody (murine), 3.0 mL/vial

Materials and Equipment Required but not Provided

- A closed system to collect and wash the cell preparation
- Empty sterile containers for fluid transfer:
- Two - 300 mL sterile containers for sterile, non-pyrogenic phosphate buffered saline (PBS)
- Two - 600 mL sterile containers for antibody labeled cells
- Sampling site couplers
- Method or instrument to perform nucleated cell counts
- Sterile, 50 mL conical centrifuge tubes
- Centrifuge or cell washer
- Laminar flow hood
- Automatic pipettor and disposable pipettes (2, 5, and 10 mL)
- Sterile, disposable syringes (3, 5, 10, and 60 mL) and needles (16 gauge or larger bore) for transfer of cells
- Liquid nitrogen storage container to store cryopreserved cells
- Controlled rate freezer and thermocouple for 5 mL cryovials
- 5 mL cryovials
- Dimethylsulfoxide, sterile, pyrogen-free
- 20 or 25% Albumin (Human), USP
- Tubing sealer
- Heparin (pharmaceutical-grade) at a concentration of 10,000 units/mL (preservative -free)
- Sterile, pyrogen-free, calcium and magnesium-free, phosphate buffered saline (PBS) 100 mL
- Water bath (37°C) to thaw cells

NOTE: Personnel processing hematopoietic stem cells may wish to consult the standards published by FAHCT (Foundation for the Accreditation of Hematopoietic Stem Cell Therapy) and AABB (American Association of Blood Banks) (3,4). Only personnel trained in the operation of the CEPRATE® SC System should perform the CD34+ cell concentration procedure. Once this procedure has been started, it should be completed without interruption. For a more detailed discussion of the CD34+ cell concentration procedure refer to the Operator Manual.

1. **Sample Collection and Preparation**

   - Collect the marrow using standard medical techniques. The time interval between harvest and start of processing should be minimized.
   - Once the marrow is harvested, the white cells must be collected and concentrated, and the red cells and plasma removed. The remaining product is called a buffy coat preparation.
   - The marrow buffy coat preparation must be washed to remove any remaining plasma. Wash the nucleated cell product twice using 500 mL RPMI 1640 media for each wash. Perform a nucleated cell count.
   - If the nucleated cell count is $\leq 60 \times 10^9$, bring the volume to 140 - 150 mL using RPMI. If the nucleated cell count is $> 60 \times 10^9$, bring the volume to 290 - 300 mL using RPMI.
   - Determine the volume and remove samples as needed for testing.

   CAUTION: Do not process more than 120×10^9 nucleated cells per column set.

2. **Antibody Incubation**

   - Determine the volume of antibody needed for incubation, based on the nucleated cell count. In the majority of patients, there will be less than 60×10^9 nucleated cells and one vial (3.0 mL) of antibody is sufficient. If there are more than 60×10^9 nucleated cells, two vials of antibody should be used.
   - Vials of antibody should be thawed undisturbed at room temperature for approximately 20 minutes.

   CAUTION: Do not shake the vials or place them in a water bath. Do not use if antibody solution is cloudy or contains precipitate. Draw the antibody into a 3 or 5 ml syringe equipped with a 16 gauge needle or larger.

   - If nucleated cell count is $\leq 60 \times 10^9$, add 0.75 mL of 20% Albumin (Human) or 0.6 mL of 25% Albumin (Human) to cells to achieve a final concentration of at least 0.1% Albumin (Human).
   - If nucleated cell count is $> 60 \times 10^9$, add 1.5 mL of 20% Albumin (Human) or 1.2 mL of 25% Albumin (Human) to cells to achieve a final concentration of at least 0.1% Albumin (Human).
   - Add thawed antibody to cells. Upon completion of this step, adjust incubation volume to 150 mL (if $\leq 60 \times 10^9$) or 300 mL (if $> 60 \times 10^9$) using RPMI.
   - Incubate cells for 25 minutes at room temperature. After 10 to 15 minutes of incubation, gently mix cells by swirling.
   - Wash cells to remove excess antibody using one liter of PBS. Bring final volume to 300 mL (if $\leq 60 \times 10^9$) or 600 mL (if $> 60 \times 10^9$) with PBS.

3. **Instrument Set-Up and Run**

NOTE: Follow the instructions in the CEPRATE® SC System Operator Manual.

- Prepare all solutions and tubing sets according to the instructions in the Operator Manual.
- Turn on the CEPRATE® Instrument power supply and select *<Load tubing>* from the *<Set-up & run>* menu. Follow the instructions to load the Tubing Set, make connections, and add Albumin (Human) to appropriate bag.
- Select *<Prime>* from the *<Set-up & run>* menu. This step starts the automated priming sequence.
- After the *<Prime>* sequence is complete, attach the bag containing the antibody-labeled, washed cells (300 mL or 600 mL final volume) to the Tubing Set with the 40µm Pall SQ40S Blood Filter.
- Select *<Run process>* from the *<Set-up & run>* menu to begin the automated concentration procedure.
- At the end of the process remove the bag containing the CD34+ selected cells from the Tubing Set. Process the cells for cryopreservation.
- Remove the Tubing and Column Sets from the instrument and discard in a biohazard container.

4. **Volume Reduction and Cryopreservation**

- Concentrate the CD34+ selected cells by centrifugation to a final cell density of between 10 - 50 x 10^6 nucleated cells/mL in a final sterile solution of 7.5% DMSO and 4% Albumin (Human) in PBS (with heparin 10 units/mL).
- Then immediately transfer the cells to 5 mL sterile cryovials for cryopreservation.
- PBS supplied in the CEPRATE® Disposable SC Kit should not be used for freezing or infusion of cells.

CAUTION: To avoid injury, do not immerse cryovials into liquid nitrogen. Always store vials only in the vapor phase above the liquid nitrogen.

STORAGE

- Store the anti-CD34 biotinylated antibody at temperatures of -70°C or lower. Allow the antibody to thaw at room temperature (undisturbed) for approximately 20 minutes just before use. **Do not refreeze the antibody once it has been thawed.**
- Store the Column Set (Avidin Column and Precolumn) and the RPMI 1640 at 2-8° C. Do not freeze the Avidin Column and Precolumn; freezing may cause the columns to crack. If columns are frozen, do not use.
- Store all other components at room temperature (15-30° C).

REFERENCES

1. Auditore-Hargreaves, K, Heimfeld S. and Berenson, RJ. Selection and Transplantation of Hematopoietic Stem and Progenitor Cells. Bioconjugate Chem. 1994, 5, 287-300.
2. Henderikx P, Moenecleay G, Van Eeckhoven E, Konigorski S, and Strobbe E. Transplantation of CD34+ Haematopoietic Progenitor Cells selected with the CellPro CEPRATE SC positive selection method: an analysis of technical and clinical results. The Mulhouse Manual. AlphaMed Press. 1996.
3. Standards for Hematopoietic Progenitor Cell Collection, Processing, and Transplantation. Foundation for the Accreditation of Hematopoietic Cell Therapy. 1996.
4. Standards for Blood Banks and Transfusion Services. American Association of Blood Banks, 17th Edition, 1996.

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