PRECAUTIONS

Potential risks related to additional Isolex processing include the risk of infusion of bacterially contaminated cells that may cause infection (see ADVERSE EVENTS; *Cell Product Sterility*). Use aseptic techniques for all procedures. All selected products should be evaluated for microbial contamination prior to reinfusion.

There is the potential for infusion of foreign proteins which are residual process components, which may evoke an immune response (see ADVERSE EVENTS; *Human Anti-Mouse Antibody (HAMA) / Human Anti-Sheep Antibody (HASA) Response*).

The Isolex Reagent Kit and disposable sets are intended for single use only. Do not reuse components. The fluid pathways of the disposable sets are sterile and nonpyrogenic. Do not use if package integrity is compromised.

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.

ADVERSE EVENTS

Engraftment Delay

Seventeen (22%) of the patients in the Isolex-processed arms had $< 2 \times 10^6$ CD34+ cells/kg in the selected product. Five of these seventeen patients received unselected (back-up) cells to provide a total dose $\geq 2 \times 10^6$ CD34+ cells/kg. The remaining twelve received a total dose $< 2 \times 10^6$ CD34+ cells/kg (selected cells), and their median time to neutrophil engraftment was not significantly different from the median time for the 63 patients in the Isolex arm who received $\geq 2 \times 10^6$ CD34+ cells/kg (11 and 10 days, respectively). However, the median time to platelet engraftment was significantly delayed for patients in the Isolex arm who received $< 2 \times 10^6$ CD34+ cells/kg compared to those who received $\geq 2 \times 10^6$ CD34+ cells/kg (14 and 12 days, respectively). No patients in the unprocessed group received fewer than 2×10^6 CD34+ cells/kg.

Impaired Hematopoietic Reconstitution

There were 5 patients in the Isolex-processed arm with evidence of impaired hematopoietic reconstitution as assessed by blood counts at one year post-transplant. Two of 26 subjects had an ANC < 1,000/µL and 4 of 31 subjects had platelets < 50,000/µL at one year post-transplant; one of these subjects had impairment of both platelet and neutrophil reconstitution. (See CLINICAL STUDIES.) An additional subject died at day 200 post-transplant without evidence of platelet engraftment.

Other Adverse Events

There were limited data collected regarding infusion-related adverse events. There were no reports of serious infusion-related adverse events in patients who received unprocessed PBPC or those who received Isolex-processed PBPC.

Cell Product Sterility

Sterility was assessed for products collected in the randomized study and Nexell sponsored Phase I and II studies[1-4]. Aliquots were cultured for aerobic and anaerobic pathogens. There were 281 Isolex 300-processed PBPC products cultured; of these, 1 product grew gram negative rods on culture. There were 186 Isolex 300i-processed PBPC products cultured of which 2, from different patients, were positive for *Propionebacterium*. There were no reports of clinical infections related to these infusions, although it should be noted that patients were receiving prophylactic antibiotics.

Human Anti-Mouse Antibody (HAMA) / Human Anti-Sheep Antibody (HASA) Response

A theoretical risk is that of infusing residual process components. Residual murine or sheep antibody may evoke an immune response; anaphylactic reactions may occur in patients with hypersensitivity to products of murine or sheep origin.

Serum samples were tested for the presence of human antibodies to murine (HAMA) or sheep (HASA) antibody. Serum samples from patients who received Isolex-processed PBPC were negative for HAMA (n = 15) and were negative for HASA (n = 13) following infusion. There were no reports of anaphylactic reactions in patients who received Isolex-processed products.

INSTRUCTIONS FOR USE

(Refer to the Operator's Manual supplied with the Isolex Magnetic Cell Selectors for Detailed Instructions For Use)

The system components, sample preparation procedure and instrument set-up depend on the specific Isolex System used. The Operator's Manual includes a detailed list of equipment and materials provided and required (see Chapter 4 for the Isolex 300 System; see Chapter 6 for the Isolex 300i System). The Operator's Manual also includes instructions for equipment and materials required, as well as preparation of solutions and samples. It is important to refer to the appropriate Isolex 300 or Isolex 300i Operator's Manual before proceeding.

The starting product for both Isolex Systems is a mobilized autologous peripheral blood progenitor cell product collected by apheresis. When the Isolex 300 System is used, some early processing steps are performed manually prior to selection, and the CD34+ cells are washed and concentrated manually after selection (see Isolex 300 Operator's Manual).

Following selection with either system, an aliquot should be obtained for microbial and other testing, and the CD34+ cell product should be frozen for later use (see Operator's Manual for details).

STORAGE

The Reagent Kit should be stored refrigerated (2 - 8°C) prior to use. Do not freeze.

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12/99

BARCODE FPO

Code 4R9734 1 Unit

Isolex Magnetic Cell Selection System

DESCRIPTION

Isolex Magnetic Cell Selection System

The Isolex Magnetic Cell Selection System consists of the following components:

- Isolex 300 or 300i Magnetic Cell Selector
- Disposable Set for Isolex 300 or 300i Magnetic Cell Selector
  This product contains dry natural rubber.
- Isolex Stem Cell Reagent Kit for either Selector containing:
  - One vial of Anti-CD34 Monoclonal Antibody
  - One vial of Dynabeads® M-450 Sheep anti-Mouse IgG
  - One vial of PR34+ Stem Cell Releasing Agent

  The packaging of this Reagent Kit contains dry natural rubber.

The Disposable Set and the Reagent Kit are intended for use only with the Isolex Selectors. Refer to the Isolex 300 and Isolex 300i Magnetic Cell Selector Operator's Manuals for a detailed description of the Isolex Selector and the Isolex disposable set.

Isolex Stem Cell Reagent Kit

Isolex Anti-CD34 Monoclonal Antibody
The Isolex antibody is an anti-human CD34 murine IgG_1 monoclonal antibody with lambda light chains. The antibody is supplied in a 2.5 mL (1.0 mg/mL) sterile, nonpyrogenic phosphate buffered saline solution. **Caution:** This product is manufactured as a colorless solution. Should discoloration occur, product should be returned to Nexell Therapeutics Inc. Slight particulate formation may develop during the shelf life of the product; this has no measurable impact on antibody function.

Dynabeads® M-450 Sheep anti-Mouse IgG
Dynabeads® M-450 Sheep anti-Mouse IgG are paramagnetic, polystyrene beads with affinity purified sheep anti-mouse IgG covalently bound to the surface. The sterile nonpyrogenic suspension contains 10 mL of approximately 4×10^8 beads/mL in phosphate buffered saline with 0.1% Albumin (Human), USP.

PR34+ Stem Cell Releasing Agent
The PR34+ Stem Cell Releasing Agent is an octapeptide that is supplied in a 20 mL sterile, nonpyrogenic phosphate buffered saline solution.

PRINCIPLES OF OPERATION

The key steps in the positive cell selection process as described in the Operator's Manual are: *Sensitization, Capture/Rosette, Separation, and Release.*

Sensitization: In the Isolex positive selection procedure, the anti-CD34 monoclonal antibody (the primary antibody) is mixed with cells in suspension to permit binding to CD34+ cells.

Capture/Rosette: Following washing to remove the unbound antibody, Dynabeads® M-450 Sheep anti-Mouse IgG are mixed with the cell suspension. Dynabeads® M-450 have been coated with the sheep anti-mouse IgG (the secondary antibody), which recognizes the murine-derived anti-CD34 primary antibody. This creates bead-target cell rosette complexes.

Separation: A magnetic field is applied to the chamber, enabling the CD34+ cell-bead complexes to be separated magnetically from the rest of the cell suspension.

Release: Following washing in the chamber of the Isolex Disposable Set to remove non-target cells, PR34+ Stem Cell Releasing Agent is introduced to separate antibodies/beads from CD34+ cells. The beads and associated antibodies are retained within the disposable chamber by the magnetic field. The separated CD34+ cells are then washed to remove residual reagents, such as mouse and sheep antibodies, and collected.

INDICATIONS AND USAGE

The Isolex 300 and Isolex 300i Magnetic Cell Selection Systems are indicated for processing autologous peripheral blood progenitor cell (PBPC) products to obtain a CD34+ cell enriched population intended for hematopoietic reconstitution after myeloablative therapy in patients with CD34-negative tumors. (See CONTRAINDICATIONS.) Isolex processing reduces the number of non-CD34+ (non-target) cells, including tumor cells, in the autograft compared with unselected PBPC. Clinical studies have not determined whether use of the Isolex 300 or 300i systems will alter progression-free or overall survival.

It is recommended that sufficient peripheral blood be collected to provide at least 2 x 10^6 CD34+ cells per kilogram of patient body weight after CD34+ cell selection. Infusion of fewer cells has been associated with delayed time to platelet engraftment. (See WARNINGS.)

CLINICAL EXPERIENCE

Description of Clinical Studies

The safety and effectiveness of the Isolex System were evaluated in an open-label, randomized clinical study designed to detect clinically significant delays in engraftment with infusion of Isolex-selected CD34+ cells as compared to unselected mobilized peripheral blood progenitor cells (PBPC)[1].

A total of 189 patients with stage II, III, or IV breast cancer who were candidates for high-dose chemotherapy with autologous PBPC rescue were enrolled. Subjects were eligible for randomization if they achieved adequate mobilization (≥ 20 CD34+ cells/µL peripheral blood); 158 patients (84%) met this criterion. A total of 142 were randomized; 76 patients were allocated to receive Isolex-processed PBPC and 66 to receive unselected PBPC. One subject, randomized to Isolex processing, relapsed prior to transplantation; thus engraftment results are reported for 75 patients in the Isolex arm. The protocol also required a minimum of 5 x 10^6 CD34+ cells/kg in the PBPC product in order to undergo Isolex-processing. Sixty-six (87%) of the 76 patients in the Isolex-processed arm had 5 x 10^6 CD34+ cells/kg collected. Ten of the 75 patients (13%) who were transplanted received back-up (unprocessed) PBPC.

Engraftment

The median time to neutrophil engraftment was the same for both arms, but the median time to platelet engraftment was longer for the Isolex arm (Table 1). Also, the Kaplan-Meier curves for time to neutrophil and platelet engraftment were statistically different based

on the log-rank test (more rapid time to engraftment in the control group). Neutrophil engraftment was defined as the first of three consecutive days where ANC > 500/µL; platelet engraftment was defined as the first of three consecutive days where platelets ≥ 20,000/µL without transfusion support.

The 95% confidence interval for the difference between the median time to recovery for unprocessed and Isolex-processed groups indicate that there is a potential 0.0 to 1.0 day delay in median time to neutrophil and a 1.0 to 3.0 days delay in median time to platelet engraftment for patients in the Isolex-processed arm.

Table 1
Engraftment Characteristics

	Unprocessed n = 66	Isolex-Processed n = 75
Median CD34+ Cell Dose	4.9 x 10^6/kg	3.3 x 10^6/kg
Median Days to ANC ≥ 500/µL* (95% CI) (Range)	10 (9-10) (8-20)	10 (10-11) (8-16)
Median Days to Platelets ≥ 20,000/µL* (95% CI) (Range)	10 (9-10) (6-60)	12 (11-12) (5-38**)

* Kaplan-Meier estimate
**Not included is one patient who died at day 200 post-transplant without platelet engraftment.

Post-transplant parameters did not show significant differences between the two study arms with regard to days of hospitalization, days of antibiotic therapy and platelet transfusion support required. The difference in the requirement for red blood cell transfusions in the Isolex arm (median of 5.2 RBC units transfused/patient) vs. the unprocessed arm (median of 4.4 RBC units/patient) was significant (p = 0.04).

Platelet engraftment (≥ 20,000/µL) was not documented for one patient who died of progressive disease at day 200 post-transplant.

Limited data are available regarding hematopoietic function at one year post-transplant. All patients in the unprocessed group with one year data (n = 34) had ANC ≥ 1,000/µL, while 2 of 26 patients in the Isolex-processed group had ANC < 1,000/µL at one year. Both patients achieved ANC > 1,500/µL by 15 months post-transplant; one had received unmanipulated PBPC 10 days post-transplant due to infection. All patients in the unprocessed group with one year data had ≥ 50,000 platelets/µL at one year (n = 39), while 4 of 31 patients in the Isolex-processed group did not. None of these patients required platelet transfusions.

Long Term Follow-up

There was no significant difference in the proportion of patients with infection of any severity (56% of unprocessed vs. 67% of Isolex-processed patients) during the first year post-transplant. However, the proportion with moderate or severe infections (33% vs. 53%) in the first year post-transplant was higher in the Isolex arm (p = 0.03). Only one patient was reported with life threatening infections; this was a control patient who received unselected PBPC. Laboratory studies to assess the adequacy of late immune reconstitution were not performed.

The Kaplan-Meier estimates of the median time to progression were 430 days and 398 days in the Isolex and unprocessed arms respectively. The 1-year mortality rates were 18% and 11%, for the Isolex and control arms, respectively. The median survival had not been reached.

DEVICE PERFORMANCE

Composition of Enriched CD34+ Cell Products

Processing of mobilized autologous apheresis products from patients reduced the total number of CD3+ and CD19+ cells by greater than 1,000-fold and 100-fold, respectively, as assessed by immunofluorescence. Device performance parameters obtained from apheresis samples used in the study conducted in patients with breast cancer are summarized in Table 2. The median CD34+ cell recovery (yield) was similar for the two devices, 45% (30%-64%, 25th and 75th percentiles, n=50) and 54% (37%-68%, 25th and 75th percentiles, n=82) for Isolex 300 and Isolex 300i, respectively. The median proportion of CD34+ cells in the processed product (purity) was 90% (80%-96%, 25th and 75th percentiles, n=50, Isolex 300 and 80%-95%, 25th and 75th percentiles, n=82, Isolex 300i) for both device configurations.

Table 2
Device Performance Summary

	Isolex 300 System			Isolex 300i System		
	Pre-selection	Post-selection	Log Depletion	Pre-selection	Post-selection	Log Depletion
TNC x 10^8						
Median	201	0.8	2.4	220	1.4	2.3
Range	(35-673)	(0.2-9.5)	(1.2-3.2)	(57-938)	(0.2-12.2)	(1.4-3.1)
# Samples	50	50	50	82	82	82
CD34+ x 10^8						
Median	1.5	0.7	NA	2.4	1.2	NA
Range	(0.3-14.1)	(0.2-9.2)		(0.3-21.1)	(.02-12.1)	
# Samples	50	50		82	82	
CD3+ x 10^8						
Median	57.8	0.01	3.5	63.3	0.02	3.4
Range	(4.4-205)	(.001-.01)	(2.2-4.7)	(4.1-162)	(.001-1.8)	(1.7-4.3)
# Samples	30	30	30	54	54	54
CD19+ x 10^8						
Median	2.2	0.01	2.3	1.6	0.02	2.0
Range	(0.1-13.1)	(.001-.04)	(1.0-3.0)	(0.1-120)	(<.001-.7)	(0.2-3.6)
# Samples	21	21	21	20	20	20

Tumor Depletion

Depletion of breast tumor cells has been quantitated using immunocytochemical assays. In eight apheresis products which had been spiked with breast cancer tumor cell lines, tumor cells were reduced > 2,000-fold.

In products from patients with non-Hodgkin's lymphoma and chronic lymphocytic leukemia, tumor depletion was assessed using immunofluorescence assays to identify lymphoma or leukemia cells based on the co-expression of B-cell markers (e.g., CD5/CD19) and/or the exclusive expression of kappa or lambda light chains[2,3,4]. In the twenty procedures with quantitative results, tumor cells were depleted by greater than 200-fold, and in eleven by greater than 1,000-fold.

Tumor cells from patients with multiple myeloma were identified by the high level expression of CD38 using an immunofluorescence assay. In a retrospective analysis of twenty-six quantitative procedures, CD38 bright cells were depleted by 64- to greater than 30,000-fold (mean 4,604-fold). Twenty-one of twenty-six procedures resulted in a greater than 200-fold reduction.

The impact of tumor cell depletion on progression-free and overall survival has not been established in randomized prospective studies.

CONTRAINDICATIONS

The use of the Isolex System is contraindicated in patients whose tumors express the CD34 antigen. The CD34 antigen has been identified on malignant cells, particularly those of myeloid and

lymphoid lineage. If appropriate, tumor specimens should be screened for the presence of CD34+ expression.

Isolex-processing is not indicated for use with previously cryopreserved and thawed PBPC products. CD34+ cell recovery and viability can be significantly decreased after Isolex-processing with cryopreserved cell products.

WARNINGS

The safety of Isolex-processing in patients with unsuccessful stem cell mobilization (a circulating CD34+ cell number of < 20/µL) or with < 5 x 10^6 CD34+ cells/kg in the apheresis products prior to selection, has not been fully studied; thus, is not established.

It is recommended that sufficient apheresis product be harvested to provide ≥ 2 x 10^6 CD34+ cells/kg of patient body weight after selection (see discussion of cell recovery in DEVICE PERFORMANCE). Failure to infuse an adequate number of CD34+ cells can result in delayed engraftment of neutrophils and platelets[5], and potentially engraftment failure.

The recommended CD34+ dose has not been prospectively validated. Further, since CD34+ cell measurements have been shown to vary widely, the value should not be considered to be definitive.

If at any time the user believes that the cells necessary for engraftment remain in the non-target fraction, the non-target fraction may be collected using aseptic techniques and cryopreserved. (See Chapter 4 for the Isolex 300 System and Chapter 6 for the Isolex 300i System for specific details for collecting non-target fractions.)

Handling, processing, or storing cell products under conditions which deviate from the procedures which are specified in the Operator's Manual requires validation to ensure that such modifications will not result in inadequate CD34+ cell yield and/or purity. It is essential that routine training of all users occur at the time that device placement is performed.

Performance failures were reported at a rate of approximately 0.3% between 1995 and 1999. Performance failures may be caused by poor quality apheresis products or not adhering to the instructions for use. Therefore, it is important to follow the instructions for use in the Operator's Manual for the Isolex device and the manufacturer's recommended instructions for use of the collection device (apheresis product).

Excessive cell clumping in the apheresis product has been associated with unsatisfactory device performance (performance failures). Procedures or conditions which promote clumping should be avoided. Although the causality has not been investigated, the following situations have been observed in association with clumping and performance failure: processing of cryopreserved apheresis product (previously frozen and thawed); low cell viability (<90% viability) in the apheresis product prior to processing; elevated platelet count in the apheresis product; and elevated paraprotein level in the apheresis product.

This product contains albumin, a derivative of human blood. Based on effective donor screening and product manufacturing processes, it carries an extremely remote risk for transmission of viral diseases. A theoretical risk for transmission of Creutzfeldt-Jakob disease (CJD) also is considered extremely remote. No cases of transmission of viral diseases or CJD have ever been identified for albumin.