SUMMARY OF SAFETY AND EFFECTIVENESS

I. GENERAL INFORMATION

Device Generic Name:	Hematopoietic Stem Cell Concentration System
Device Trade Name:	Isolex® 300 Magnetic Cell Selection System and Isolex® 300i Magnetic Cell Selection System
Applicant's name and address:	Nexell Therapeutics Inc. Irvine, California 92618 USA
PMA numbers:	Reference number BP97-0001 and BP97-0001/01
Date of Panel recommendation:	July 24, 1997
Date of notice of approval to applicant:	July 02, 1999

II. INDICATIONS FOR USE

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. 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.

III. DEVICE DESCRIPTION

Device Description - Isolex® 300 System

The Isolex® 300 System is a semi-automated magnetic cell separation system designed to select and isolate CD34 positive cells, *ex vivo,* from mobilized peripheral blood using anti-CD34 monoclonal antibody and paramagnetic microspheres. The Isolex® 300 System for positive selection of CD34+ cells is a combination of devices and biological components (supplied in the Isolex® Stem Cell Reagent Kit). The devices consist of an instrument for separating paramagnetic microspheres from mobilized peripheral blood mononuclear cell (MNC) suspensions (Isolex® 300 Magnetic Cell Separator) and an associated disposable set for providing the fluid path (Isolex® 300 Magnetic Cell Separator Disposable Set).

Key components of the Isolex® 300 Magnetic Cell Separator include a primary magnet

whose adjustable position is controlled automatically, a stationary, secondary magnet, and a user interface, which consists of a keypad for operator input and a liquid crystal display for prompting the operator. The associated disposable set comprises a sterile biocompatible fluid path for the cells. Main components of the disposable set include the mixing/separation chamber, which interfaces with the primary magnet, and the secondary chamber, which interfaces with the secondary magnet.

Device Description: The Isolex® 300i System

The Isolex® 300i System is an automated magnetic cell separation system designed to select and isolate CD34 positive cells, *ex vivo*, from mobilized peripheral blood by using anti-CD34 monoclonal antibody and paramagnetic microspheres. The Isolex® 300i System for positive selection of CD34+ cells is a combination of devices and biologic components (supplied in the Isolex® Stem Cell Reagent Kit). The devices consist of an instrument for separating paramagnetic microspheres from mobilized peripheral blood MNC suspensions (Isolex® 300i Magnetic Cell Separator) and an associated disposable set for providing the fluid path (Isolex® 300i Magnetic Cell Separator Disposable Set).

The Isolex® 300i Magnetic Cell Separator instrument is an electro-mechanical magnetic cell separator/cell washer. Key components of the instrument include an array of primary magnets to capture beads and bead/cell complexes; a secondary magnet, designed to capture residual beads; a rocker module/primary magnet housing to hold the primary chamber of the disposable set; clamps to hold the disposable set manifolds; pumps to move fluid through different paths of the disposable set; a spinner that spins the spinning membrane device of the disposable set; a membrane switch keypad; a graphic LCD to control the instrument functions and monitor the procedure; pressure transducers to monitor the fluid path pressure; fluid detectors to monitor fluid levels; and a solution tower with six weight scales and bag hangers to support the bags of the disposable set.

The associated disposable set comprises a sterile biocompatible fluid path for the cells. Main components of the disposable set include the mixing/separation chamber, which interfaces with the primary magnet, and the secondary chamber, which interfaces with the secondary magnet. Other key components are the spinning membrane assembly; transfer/reservoir bags for containing the reagents, end product and waste product, and for in-process washing; sterilizing filters for filtering the incoming reagents and buffer; clamp manifolds and tubing organizers.

Isolex® Stem Cell Reagent Kit

A reagent kit, containing the biological reagents for use with either selector system, is supplied separately. The kit contains the following:

a. A murine monoclonal antibody directed against the human CD34 antigen expressed on hematopoietic progenitor/stem cells; this antigen is expressed primarily by a small percentage of bone marrow and peripheral blood mononuclear cells (MNC) however has also been identified on some malignant

cells, particularly those of myeloid and lymphoid lineage.

b. Paramagnetic microspheres coated with sheep anti-murine antibody (Dynabeads® M-450 Sheep anti-Mouse IgG). The sheep antibody coated microspheres bind murine immunoglobulin and provide the mechanism for targeting the murine antibody coated CD34+ cells for selection.

c. An octapeptide (PR34+™ Stem Cell Releasing Agent) that noncnzymatically competes for the CD34+ antibody binding site on the targeted $CD34^{+}$ cells, thus resulting in release of the CD34+ cell from the antibody-coated paramagnetic microspheres. After the PR34+ agent displaces the CD34+ cells from the bead-cell rosettes, the beads are retained by the magnet, allowing the CD34+ cells to be collected.

PRINCIPLES OF OPERATION

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

Sensitization: The murine 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+ cells-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.

IV. CONTRAINDICATIONS, WARNINGS, PRECAUTIONS

Contraindications:

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

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/Precautions:

The safety of Isolex®-processing in patients with unsuccessful stem cell mobilization, as defined in clinical studies by a circulating CD34+ cell number of <20/μL, and patients with < 5 x 10^6 CD34+ cells/kg in the apheresis product prior to selection, has not been fully studied, thus, is not established.

Adequate PBPC collection prior to processing
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 **IX. SUMMARY OF CLINICAL STUDIES; Integrated Summary of Device Performance**, Tables 5 and 6, regarding median CD34+ cell recovery). Failure to infuse an adequate number of CD34+ cells can result in delayed engraftment of neutrophils and platelets, 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.

Device failure and performance failure
Device failures have been identified through complaints reported to the manufacturer. The complaints have encompassed all three components (instruments, disposable sets, and biologic reagents) of the system. The information provided by the sponsor covers the period between Jan. 1995 and Dec. 1997. The sponsor states that there have been no reports of a loss of an apheresis product due to device failures. As specified in the Operations Manual, the appropriate procedures for initial self tests, including systems initialization and disposable set installation check, should always be performed.

Information was provided for complaints received over a two year period during which 163 Isolex® 300i Magnetic Cell Separator instruments (51 under investigational use and 112 under commercial use) and 196 Isolex® 300 Magnetic Cell Separator instruments (163 under investigational use and 60 under commercial use) were distributed and in use. The rate of complaints was higher, on a per-instrument basis, for devices under investigational use as compared to commercial use. The rate of complaints was higher for the Isolex® 300i Magnetic Cell Separator instrument (15% for commercial use) as compared to Isolex® 300 Magnetic Cell Separator instrument (2% for devices in commercial use). The sponsor attributes this to the greater complexity of the Isolex® 300i Magnetic Cell Separator. Complaints related to the Isolex® 300i Magnetic Cell Separator were categorized under the following: workmanship, mechanical problems with the rocker, cable, fluid detector, clamp, pressure transducer, and/or magnet, software, and user/training. Complaints related to the Isolex® 300 Magnetic Cell Separator were categorized under the following: workmanship, mechanical problems with the IV pole and/or magnet.

Information was provided for complaints received over a 2 year period covering

distribution of 3132 Isolex® 300i Disposable Sets (1693 in investigational use and 1493 in commercial use) and 3032 Isolex® 300i Disposable Sets (2140 in investigational use and 892 in commercial use). As noted above, the rate of complaints was higher for products used in an investigational setting as compared to commercial use and for the Isolex® 300i Disposable Set as compared to the Isolex® 300 Disposable Set (2% vs. 0%). It should be noted that the Isolex® 300i Disposable Set is more complex than the Isolex® 300 Disposable Set, with regard to the number of bonds and inclusion of additional components (spinning membrane, buffer filter). The complaints received for the Isolex® 300i Disposable Set were categorized as: set defect, buffer filter, spinning membrane, installation problems and user/training. The complaints received for the Isolex® 300 Disposable Set were categorized as set defects.

The complaints received regarding the Isolex® 300i and the Isolex® 300 Reagent Kits included expiration dating, particulates in the antibody product, and clumping and/or poor yield in the selected apheresis product. The rates estimated for clumping or poor yield (based on number of disposable sets distributed) ranged from 0.2-2.0% and were higher for the Isolex® 300 Reagent Kits.

Performance failures, observed in clinical studies and received as complaints to the manufacturer, have been reported a rate of approximately 0.3% between 1995 and 1999. Performance failures may be caused by poor quality apheresis products or failure to adhere 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 apheresis collection device. It is essential that routine training of all users occur at the time of device placement and that operators have read and comply with the instructions in the Operator's Manual, which provides specific details regarding the use of the system.

If at any time the user believes that the cells necessary for engraftment remain in the non-target fraction, the non-target fraction should be collected using strict 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.) In addition, if recovery of CD34+ cells is reduced as a result of device failure or a performance failure e.g., significant clumping or low viability, the collection of additional apheresis products may be necessary.

Handling, processing, or storing cell products under conditions which deviate from the procedures specified in the Operator's Manual, including cryopreservation of pre-processed PBPC, requires validation to ensure that such modifications will not result in inadequate CD34+ cell yield and/or purity.

Excessive cell clumping in the apheresis product has been associated with unsatisfactory device performance. 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.

Contamination of the PBPC Product

Additional processing increases the risk of bacterial contamination of the PBPC product. (see **Section VII.** ADVERSE EFFECTS OF THE DEVICE ON HEALTH; Bacterial Contamination of the PBPC Product). Use aseptic techniques for all procedures. There is typically a reasonable time period (weeks) between the collection of PBPC and subsequent re-infusion of the thawed PBPC product; during this period, it is recommended that cultures of the CD34+ selected cells be performed and results obtained prior to the clinical use of selected products. The routine use of microbiological assays (cultures) for detection of contamination with infectious agents (bacterial and fungal) is recommended to identify contaminated products.

Immunogenicity

There is the potential for infusion of foreign proteins that are residual process components (see **Section VII.** POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH; Potential for Exposure to Immunogenic Xenogeneic Proteins). Patients should be evaluated for history of allergy to murine or ovine (sheep) products. Patients should be informed that xenogeneic proteins are components of this procedure and, if sensitized, that the safety and efficacy of murine or ovine-based *in vitro* diagnostics and *in vivo* diagnostic and therapeutic agents used in the future may be altered.

Other

The Isolex® Stem Cell Reagent Kit, the Isolex® 300 Magnetic Cell Separator Disposable Set and the Isolex® 300i Magnetic Cell Separator Disposable Set 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.

V. ALTERNATIVE PRACTICES AND PROCEDURES

Bone marrow and peripheral blood progenitor cell transplantation are medical procedures that are performed under the practice of medicine. Published literature supports the use of autologous progenitor cell transplantation as a salvage therapy for aggressive non-Hodgkin's lymphoma and as salvage therapy for acute leukemia (where no allogeneic donor is available). Autologous PBPC transplantation is also commonly used in support of dose-intensive chemotherapy for the treatment of children with solid tumors and is being investigated for it's utility in the treatment of multiple myeloma and advanced breast cancer.

Use of unselected PBPC products for autologous transplantation is an acceptable

alternative to use of Isolex-selected PBPC, as there has been no evidence that Isolex-selection improves disease-free or overall survival.

There are currently no approved products for the enrichment of CD34+ cells obtained for autologous progenitor cell transplantation. There was one device (the CELLPRO CEPRATE® SC Stem Cell Concentration system) which was approved in 1996 for the processing of autologous bone marrow intended for hematopoietic support. In this setting, infusion of the CD34+ enriched cell population resulted in a reduction in the incidence of DMSO-infusion-related toxicity. The CEPRATE® SC System was approved in 1998 for use with autologous mobilized peripheral blood progenitor cells. It was indicated in this setting for enrichment of CD34+ cells and reduction of tumor cells contaminating the PBPC product. This device is no longer being marketed.

VI. MARKETING HISTORY

Commercial distribution began in the E.U. in June 1996 after the Isolex® 300i Magnetic Cell Separator and Disposable Set had been CE marked in accordance with the Council Directive 93/42/EEC concerning medical devices. The PR34+™ Peptide reagent kit had been previously CE marked for E.U. distribution in January 1996. The Therapeutic Products Program of Health Canada issued a medical device license for the sale of the Isolex® 300 and Isolex® 300i (#2816) cell selection systems on May 10, 1999.

In June 1998, the French Ministry of Health Microbiological Safety Expert Group notified Nexell that a viral safety evaluation was needed for the CE marked Isolex® Reagent Kit in order to be included in their positive list for devices that include materials of bovine or ovine origin. This was a new requirement due to changes to the French regulatory law for devices. In June 1998, Nexell submitted viral safety documentation and in July 1998, the Isolex® Reagent Kit gained viral safety approval and it was included in the positive device list published by the French MOH.

Export of the Isolex® 300i System to the E.U. for commercial distribution was under Section 801(e) of the Food, Drug and Cosmetic Act. Under the same regulation, the Isolex® 300i System was exported to Australia for clinical investigation. Aside from the situation in France noted above, no countries have withdrawn any Isolex® 300 or 300i products from the market or from clinical studies for reasons related to the safety or lack of effectiveness of the device.

VII. POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Loss of Progenitor Cells Leading to Delays in Time to Engraftment

Isolex®-processing results in an absolute loss of all cells, including approximately 50% loss of the CD34+ cells collected. In the major efficacy study, the median loss of CD34+ cells after Isolex® processing was 54% (36%-70%, 1st and 3rd quartiles) for the Isolex®

300 System and 46% (32%-63%, 1st and 3rd quartiles) for the Isolex® 300i System. . Failure to infuse an adequate number of CD34+ cells (<2 x 10^6 CD34+ cells/kg) can result in delayed engraftment of neutrophils and platelets, and potentially engraftment failure.

In the major efficacy study, none of the 66 subjects in the unprocessed (control) group had fewer than 2 x 10^6 CD34+ cells/kg collected or reinfused. Seventeen of 75 patients (22%) in the Isolex®-processed arm had < 2 x 10^6 CD34+ cells/kg in the selected product. Twelve of these 17 patients were reinfused with a total dose of < 2 x 10^6 CD34+ cells/kg (the remaining 5 subjects received "back-up" unprocessed PBPC products). The median time to neutrophil engraftment in these 12 patients was delayed as compared to those receiving ≥2 x 10^6 CD34+ cells/kg in the Isolex® arm (11 days vs. 10 days) and as compared the patients who received unprocessed PBPC (11 days vs. 10 days). The median time to platelet engraftment was significantly delayed for the 12 patients who received <2 x 10^6 CD34+ cells/kg as compared to the 63 subjects receiving ≥2 x 10^6 CD34+ cells/kg in the Isolex® arm (14 days vs. 12 days). The median time to platelet engraftment was also significantly delayed for the 12 patients who received <2 x 10^6 CD34+ cells/kg as compared to the 66 patients who received unprocessed PBPC (14 days vs. 10 days).

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, while there were no patients with evidence of impaired engraftment at one year in the control (unprocessed) arm. The dropout rate was high, thus less than half of the study population was evaluable for one-year engraftment. Among those randomized to the Isolex®-processed arm, 2 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. In addition to these five, there was one subject who died at day 200 with no evidence of platelet engraftment. The clinical consequences of impaired engraftment were not well documented in case report forms. However one subject had an interruption of adjuvant Tamoxifen therapy as a result of persistent cytopenias.

Impaired engraftment was also identified in a multicenter single arm supportive study conducted in subjects with B-cell malignancies. A detailed review of engraftment data was conducted by FDA for the initial 71 subjects enrolled, all of whom received Isolex® 300-processed PBPC. Impaired engraftment was noted in nine patients (9/71 [11%]), seven of whom received bone marrow in addition to PBPC. Two of the nine subjects had delayed engraftment of both neutrophils and platelets and the remaining six had delayed platelet engraftment. The manufacturer has also identified subjects with delayed engraftment in this study among the 49 subjects who received Isolex® 300i-processed PBPC. The reported rate of delayed engraftment was lower in subjects who received Isolex® 300i-processed PBPC

Cell losses as a result of device or performance failure

There was no loss of PBPC product reported due to device failure. It should be noted that the recommended procedure for processing requires a "pre-test" of the system prior to introduction of patient materials. The potential for loss of PBPC material intended for patient use may be higher if recommended pre-test or self-test procedures are not followed.

Performance failure is defined as the failure of the final CD34+ selected product to meet intended specifications for purity, viability, and CD34+ cell yield. There were 23 reports of performance failure received between 1995 and 1999 (performance failure rate approximately 0.4%). No causal relationship has been established, however, the following conditions were reported in association with performance failure: use of clumped apheresis product, 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.

There was a single instance of enrichment of leukemic cells following Isolex-processing that was observed in a subject with CLL. This finding occurred with an earlier version of the Isolex 300 device in which chymopapain, rather than the PR peptide, was used as a releasing agent.

Bacterial Contamination of the PBPC Product

Additional processing increases the risk of bacterial contamination of the PBPC product. Infusion of such a product may result in clinical infection and potentially serious morbidity and mortality. Conditions that increase the risk of bacterial contamination in the final, processed product have not been identified. (See **Section III. Warnings/Precautions**; Contamination of the PBPC Product).

One of 281 Isolex 300-processed PBPC products grew gram negative rods on culture. Two of 186 Isolex 300i-processed PBPC products, from different patients, were culture 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.

POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH

Potential for Exposure to Immunogenic Xenogeneic Proteins

There is the potential for infusion of foreign proteins that are residual process components. In a subset of patients tested in the major efficacy study, 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. (See **Section III. Warnings/Precautions**; Device failure and performance failure).

VIII. SUMMARY OF PRECLINICAL STUDIES

DISPOSABLE SETS

Hemolysis Test

The hemolytic potential of the 300 and the 300i Disposable Sets on rabbit whole blood was assessed by incubation of a 0.9% saline extract for 4 hours at 37 C. Spectrophotometric analysis of the amount of undiluted hemoglobin in the supernatant showed no lysis of erythrocytes.

USP Rabbit Pyrogen Test

Intravenous injection of a saline extract of the 300 Disposable Set (10 mL/kg) into rabbits was non-pyrogenic.

Cytotoxicity Study

Extracts of the 300 Disposable Set (using Minimum Essential Medium) were incubated in monolayer with L-929 mouse fibroblast cells for 48 hours. No changes in cell morphology were evident. A similar test performed on the 300i Disposable Set resulted in 10% cell lysis and intracellular granulation [slight reactivity] in 30% of the cells in one of three samples.

Acute Intracutaneous Reactivity Test in Rabbits

Rabbits intracutaneously injected with 0.2 mL of extracts of the 300 or 300i Disposable Set (sodium chloride or cottonseed oil) displayed no dermal irritation when evaluated at 24, 48, and 72 hours post-dose.

USP Systemic Toxicity in Mice

Mice that were intravenously injected with 50 mL/kg of extracts of the 300 or 300i Disposable Set (sodium chloride or cottonseed oil) exhibited no systemic toxicity at 4, 24, 48, or 72 hours postdose.

Delayed Contact Sensitization Study in Guinea Pigs

Sensitization Phase - Guinea pigs were intradermally injected with 0.1 mL of Freund's Complete Adjuvant, 0.1 mL of extracts of the 300 or 300i Disposable Set (sodium chloride or cottonseed oil), or extract vehicle. One week later, topical application of sodium lauryl sulfate and an occlusive patch with 0.3 mL of test extract to all sites occurred and remained in place for 48 hours.

Challenge Phase - Two weeks after the placement of the patch, another occlusive patch containing 0.3 mL of text extract of vehicle was topically applied for a 24-hour period.

No sensitization reaction occurred at 24, 48, 72, or 96 hours after patch removal.

9069N PEPTIDE [PR34+ STEM CELL RELEASING AGENT]

Hemolysis Test

The hemolytic potential of the 9069N Peptide on human whole blood was assessed by

incubation of 0.1, 1.0, or 3.0 mg/mL of 9069N for 1 hour at 37° C. Spectrophotometric analysis of the amount of undiluted hemoglobin in the supernatant showed no lysis of erythrocytes.

In Vitro Chromosomal Aberration Evaluation
CHO cells exposed to 0.5, 1.5, or 5 mg/mL of 9069N with and without the S9 microsomal fraction did not display any increase in chromosomal damage compared to negative controls.

In Vivo Micronucleus Assay
Mice intravenously injected with 9069N at 1.5, 5, or 15 mg/kg/day for 3 days were killed on day 4, followed by isolation and examination of bone marrow cells. The frequency of micronucleated polychromatic erythrocytes (PCEs) was not increased compared to negative controls.

Acute Intravenous Toxicity in Rats
Rats were intravenously injected with 9069N at 0, 0.015, 0.15, 1.5, 15, or 150 mg/kg, and killed 14 days later. No clinically meaningful changes were noted compared to controls. The no-observable-adverse-effect-level was 150 mg/kg/dose.

Acute Intravenous Toxicity in Rabbits
Rabbits were intravenously injected with 9069N at 0, 0.015, 0.15, 1.5, 15, or 150 mg/kg, and killed 14 days later. No clinically meaningful changes were noted compared to controls. The no-observable-adverse-effect-level was 150 mg/kg/dose.

Guinea Pig Dermal Sensitization Study in Guinea Pigs [Magnusson & Kligman method]
Induction Phase - Guinea pigs were intradermally injected with 9069N at 3 mg/site, followed by topical administration of 0, 10, or 30 mg/mL on day 8.

Challenge Phase - On day 22, 10 or 30 mg/mL of 9069N was topically applied.

No sensitization reaction occurred.

DYNABEADS M-450 SHEEP ANTI-MOUSE IgG

Hemolysis Test
The hemolytic potential of the coated beads on human whole blood was assessed by incubation of 2.25×10^6 beads from 1-4 hours at 37°C. Spectrophotometric analysis showed no hemolysis of erythrocytes.

Cytotoxicity Study
Sterile filter paper disks saturated with the beads were placed in fluid medium wells or in agar diffusion wells with L-929 mouse fibroblast cells for 24 hours. The fluid medium wells were stained with 2% crystal violet and the agar plates were evaluated microscopically for cellular toxicity. No cytotoxicity was evident.

Acute Intracutaneous Reactivity Test in Rabbits
Rabbits intracutaneously injected with extracts of the beads (saline. alcohol, or cottonseed oil) displayed no dermal irritation when evaluated at 24, 48, and 72 hours postdose.

Acute Intramuscular Reactivity Test in Rabbits
Rabbits were intramuscularly injected with 4.18×10^6 beads, followed by kill on day 7. No adverse effects were observed.

Acute Intravenous Toxicity in Rats
Rats were singly intravenously injected with 9.6×10^4 beads/kg (killed after 14 days) or 8.3×10^8 beads/kg (killed after 14 or 42 days). Clinical pathology parameters were evaluated and histopathology was performed. No adverse effects were seen. As expected, the beads were taken up by the reticuloendothelial cells in various tissues [lung, liver, spleen].

Delayed Contact Sensitization Study in Guinea Pigs
Sensitization Phase - Guinea pigs were intradermally injected with 0.1 mL of Freund's Complete Adjuvant, 0.1 mL of extracts of the disposable set (sodium chloride or cottonseed oil), or extract vehicle. One week later, topical application of sodium lauryl sulfate and an occlusive patch with 0.3 mL of test extract to all sites occurred and remained in place for 48 hours.

Challenge Phase - Two weeks after the placement of the patch, another occlusive patch containing 0.3 mL of text extract of vehicle was topically applied for a 24-hour period.

No sensitization reaction occurred at 24, 48, 72, or 96 hours after patch removal.

Testing of Uncoated Beads
The following tests were performed using uncoated, nonextracted beads: cytotoxicity; hemolysis, mutagenicity test; intravenous toxicity in mice; intracutaneous toxicity in rabbits; intramuscular toxicity in rabbits; and dermal sensitization test in guinea pigs. No untoward effects were observed.

IX. SUMMARY OF CLINICAL STUDIES

A. Major Efficacy Trial

The use of the Isolex® 300/300i System in autologous transplant patients is supported by data from a single, multicenter, randomized clinical trial, conducted at eleven institutions, with seven sites enrolling > ten patients. This randomized study was designed to evaluate recovery from myeloablative chemotherapy in high-risk breast cancer patients randomized to receive either PBPC or isolated autologous $CD34^+$ cells (selected PBPC) for hematopoietic rescue following high dose chemotherapy. The study was initiated in October 1994 with the Isolex® 300 device and amended several times, including December 1995 (Isolex® 300i device to be used for selection) and October 1996 (study

endpoints and analytic plan revised).

Protocol Title: 92004-302103 "Peripheral Blood Stem Cells (PBSC) or Isolated CD34+ Cells from Mobilized Peripheral Stem Cell Collections for Hematologic Rescue of Advanced Breast Cancer Patients Treated with High- Dose Chemotherapy"

Objectives:
The initial protocol specified two primary endpoints: a)[Frequency and severity of] unusual or unexpected side effects compared to control and b)Time to engraftment [days to first of 3 with ANC>500 and platelets >20K]

The endpoints were modified as a result of teleconference discussions between the Agency and Nexell. These modifications were made after patients received their PBPC transplants but prior to any analyses of the data. The original objectives were retained and additional objectives were identified. Also, the first objective identified in the earlier version of the study, the comparison of infusion-related toxicity, was identified as a secondary endpoint in the revised statistical plan submitted on Nov. 22, 1996. The revised endpoints were:

Primary efficacy endpoint: No delay in myeloid engraftment. In the analysis of the study, the lack of a clinically important delay in neutrophil engraftment would be demonstrated by excluding a delay of more than 3 days, based on 95% confidence intervals, in the median time to neutrophil engraftment for patients receiving CD34+ selected as compared to those who received unselected cells. In this analysis, neutrophil engraftment was defined as the first of 3 consecutive days with an ANC >500.

Secondary endpoints:
- Time from infusion to an absolute neutrophil count of >1,000/μl (the first of 2 consecutive days when the neutrophil count was >1,000 cells/μl)
- Time to first of 3 consecutive days of a platelet count of ≥20,000/μl, ≥50,000/μl and ≥100,000/μl (in the absence of recent platelet transfusions)
- Incidence of infusional toxicities
- Incidence of infectious episodes
- Mortality

Study Design: Multicenter, randomized, open-label, controlled (unselected PBPC) study conducted in the U.S. and Europe.

Eligibility Criteria :
Women with high-risk Stage II, III, or metastatic adenocarcinoma of the breast, who were eligible for institutional high-dose chemotherapy and PBPC transplant protocols*, and ambulatory with ECOG performance status 0-1 or Karnofsky 100-70%, age 18-60 years,

*At Yale, all study participants underwent a tandem transplant procedure. Entry into this study was restricted to the second stem cell transplant procedure.

adequate organ function. Patients with CNS disease, including CNS metastases, serious localized or systemic infection, and those who were seropositive for HIV were excluded. Following registration, only those subjects with evidence of successful mobilization (>20 CD34+ cells/μl in peripheral blood) were eligible for randomization.

Randomization plan:
Randomization lists for each center were generated by Nexell and provided to each of the sites. Subjects were randomized following mobilization but before leukapheresis. There were no stratification factors other than center.

Treatment plan:
Patients were mobilized using chemotherapy followed by G-CSF or using G-CSF alone, with a minimum requirement of ≥20 CD34+ cells/μl to continue on the protocol. Patients who achieved the minimum mobilization requirement of 20 CD34+ cells/μl in peripheral blood were randomized to the control group (unselected PBPC) or the test group (Isolex® selected CD34+ cells).

Collection Phase: PBPC were collected by leukapheresis with a minimum collection target of 6.5 x 10^6 CD34+ cells/kg for patients in the test group, and 2.5 x 10^6 CD34+ cells/kg for patients in the control group. The target collection for the test group was based on: 1) the requirement for a back-up unmanipulated product (containing 1.5 x 10^6 CD34 cells/kg) which would serve as a source of "backup" stem cells in the event of delayed engraftment and 2) the assumption that approximate recovery after selection would be 50% (>2 x 10^6 CD34 cells/kg after selection). For patients randomized to Isolex-300/300i selection, the protocol required that a total of ≥5 x 10^6 CD34+ cells/kg be available for processing; patients with fewer than 5 x 10^6 CD34+ cells/kg were to receive unprocessed cells.

The leukapheresis products were generally not pooled before Isolex®-processing, however on occasion, two sequential leukopheresis products were pooled and processed as a single unit. On these occasions, the earlier leukopheresis product was refrigerated prior to selection. Following processing, the selected cell product was cryopreserved. Unselected products were cryopreserved after collection.

Transplantation: Patients underwent myeloablative chemotherapy according to the standard practice of the study site. Following dose-intensive/myeloablative chemotherapy, patients received unmanipulated PBPC (control group) or CD34+ cells isolated from mobilized peripheral blood cell collections with the Isolex® 300/300i System (test group) and were to receive daily G-CSF until the ANC was >1000/μl for 3 days.

Patient Assessments
- Baseline assessments included a history and physical examination, tumor extent (sites of disease) and performance status at study entry.
- During the mobilization phase, leukocyte count and differential and the concentration

of CD34+ cells in peripheral blood were assessed. The PBPC products were assessed for viability, CD34+ cell concentration, CFU, and lymphocyte markers; selected products were assessed for these parameters after Isolex 300/300i processing but prior to cryopreservation.

- Vital signs were to be taken before and after PBPC or selected cell product infusion.
- Immediate post-transplant period: After infusion, daily complete blood counts were to be obtained until neutrophil counts were >500 cells/μl and platelets counts were >20,000/μl. Information regarding infection, transfusion requirements, concurrent medications, and adverse experiences were also collected in the immediate post-transplant period (generally until discharge).
- Long-term follow-up included hematologic assessment with complete blood count data and performance status collected monthly for 6 months, then approximately every six months. Tumor response data, presence or absence of clinically significant organ impairment, presence or absence of secondary malignancy/myelodysplastic syndrome and survival information collected approximately every 6 months for 2 years then annually up to 5 years. Information regarding other events was collected at the investigator's discretion.

Results:

Patient Disposition

Of 189 patients enrolled in the study, 142 were successfully mobilized and randomized to one of the treatment groups. Failure to achieve adequate mobilization was the most frequent reason for not completing the study. Seventy-six patients were randomized to receive Isolex®-selected CD34+ cells (test) and 66 were randomized to receive unmanipulated PBPC (control). Sixty-six (87%) of the 76 patients in the test arm had adequate cells collected. One subject, who was randomized to the test arm, relapsed prior to transplantation; thus transplant and engraftment information were reported for 75 patients in the test arm.

Baseline variables and demographic information

Information regarding the subjects who were randomized and who form the basis of the intent-to-treat population for the major efficacy study are summarized, by device, in the table below. The subjects enrolled in the early portion of the study, when the Isolex 300 device was used, had more advanced disease, with 75-80% of patients with stage IV disease. In the latter part of the study, when the Isolex 300i device was used, the population was more evenly balanced between those with high-risk, local-regional disease and those with metastases at entry. The majority of the patients (60-80%) had received one or two prior chemotherapy courses and approximately one-third to one-half had received prior radiotherapy. The population enrolled was relatively young and included no elderly (age ≥ 65 years) women.

Table 1 Baseline variables and demographics					
Variable		**Isolex 300 Device**		**Isolex 300i Device**	
		Unprocessed (n=22)	Isolex-300 processed (n=28)	Unprocessed (n=44)	Isolex-300i processed (n=48)
Stage	IIB	5%	8%	18%	14%
	IIIA	14%	7%	14%	23%
	IIIB	5%	7%	27%	13%
	IV	77%	79%	41%	50%
Age (yrs)	Median (range)	46 (31-62)	45 (28-60)	43 (29-63)	45 (27-60)
# Prior Radiotherapy	0	55%	43%	68%	67%
	1	41%	43%	27%	31%
	≥2	4%	14%	5%	2%
# Prior ChemoRx	1-2	59%	72%	79%	79%
	3	32%	14%	7%	8%
	≥4	9%	14%	14%	13%
Preparative Regimens	Carb/cyclo/thio	9%	14%	46%	43%
	Carb/cyclo/etop	27%	25%	14%	15%
	Cyclo/melp/mito	41%	43%	7%	6%
	Thiotepa	14%	7%	7%	11%
	Cyclo/thio	0%	0%	14%	9%
	Bu/melp/thio	0%	0%	9%	11%
	Other	10%	11%†	4%	6%‡
# apheresis procedures performed§	1	23%	4%	37%	9%
	2	55%	32%	44%	44%
	3	9%	25%	9%	27%
	4	9%	32%	9%	9%
	≥5	5%	7%	0%	18%

Efficacy Analyses

Engraftment profile

The median PBPC cell dose infused in the unprocessed arm was 4.9 x 10^6 CD34 cells/kg and for the Isolex®-processed arm was 3.3 x 10^6 CD34 cells/kg. The median time to neutrophil engraftment was 10 days for the patients in the unprocessed group and 10 days in the Isolex®-processed group. There was no difference in the median day to neutrophil engraftment between the two study arms. However, based on the upper limit of the 95%

† Melphalan alone or cyclophosphamide plus melphalan

‡ Carboplatin + mitomycin + thiotepa or cyclophosphamide + doxorubicin + etoposide

§ Note that target collection requirements were different between the two study arms; patients randomized to no processing were required to collect ≥2.5 x 10^6 CD34+ cells/kg while those randomized to Isolex processing were required to collect ≥6.5 x 10^6 CD34+ cell/kg

confidence interval for the difference in the medians, there could be a delay in median time to neutrophil engraftment of up to one day for patients in the Isolex® processed arm (95% CI for the difference in the medians, 0 to +1 days). The median time to platelet engraftment was 10 days in the control group and 12 days in the Isolex®-processed group. There was a 2 day delay in the median time to platelet engraftment for patients in the Isolex® processed arm as compared to the control group; the 95% confidence interval for the difference in the medians was +1 to +3 days later for Isolex®- processed arm. These delays in the median time to neutrophil and platelet engraftment were judged not to be clinically important. A comparison of the Kaplan-Meier curves for the time to neutrophil engraftment and for time to platelet engraftment showed that time to engraftment was significantly shorter (log-rank tests) for the patients who receive unselected PBPC as compared to those who receive Isolex®-processed cells.

Table 2
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†	10	10
(95% CI)	(9-10)	(10-11)
Median Days to Platelets ≥ 20,000/μL†	10	12
(95% CI)	(9-10)	(11-12)

† Kaplan-Meier estimate

Immediate post-transplant course

There were no significant differences between the two study arms with regard to days of hospitalization, days of antibiotic therapy, and platelet transfusion support required. However, there was a greater requirement for red blood cell transfusions in the Isolex®-processed arm (median of 5.2 RBC units/patient) as compared to the control arm (median of 4.4 RBC units/patient), which was statistically significant.

Infectious complications as a surrogate for immune reconstitution

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. Laboratory studies to assess the adequacy of late immune reconstitution, e.g., immunoglobulin levels, T cell proliferative responses, responses to recall antigens, were not performed.

Impact on infusion-related adverse events

The incidence of adverse events temporally associated with cell infusion was similar between the Isolex-processed group and the unprocessed group. However, it should be noted that the total volumes for the PBPC product (processed and unprocessed) and the

rates of infusion were not controlled in this trial. There were significant variations between sites, and in some instances within a single site, with regard to the final volume and rate of infusion of Isolex®-processed PBPC products. In addition, monitoring for toxicities, including assessment of vital signs and other specific signs and symptoms at regular intervals during and immediately post-infusion, was not standardized at study sites; case report forms collected information only as pre- and post-infusion data. There were no differences between the study arms with regard to toxicities identified as "infusion-related". The investigators attributed most of the toxicities and all serious toxicities occurring within 24 hours of infusion to the myeloablative chemotherapy regimen and other medications, rather than to the PBPC product. The basis for this determination was not provided in the application and there were no prespecified criteria in the protocol for assessing attribution of observed toxicities to specific interventions.

Progression-free and overall survival

Kaplan-Meier estimates of the time to relapse and time to death showed no significant difference between patients who received unselected PBSC and those who received cells selected with the Isolex® 300/300*i* device. The median time to relapse in the control group was 398 days and the median for the Isolex® processed group was 430 days ($p = 0.31$). The one-year mortality rates were 18% and 11% for the Isolex® and control arms, respectively; median survival had not been reached.

Device Performance characteristics (including device and performance failures):

The Isolex® 300/300i devices were designed to go through a self-test process at the initiation of each procedure. Thus, the sponsor stated that most mechanical and structural device failures were detected prior to the introduction of any patient PBPC product to the system. The sponsor has not identified any subject in whom a mechanical/structural device failure led to loss of the selected product in this study (see **Section IV. Warnings/Precautions**). There were performance failures reported in this study which included clumping in the PBPC product leading to inadequate cell yield.

Device performance parameters assessed in the randomized study included purity (percentage of CD34+ cells/TNC), viability (viable cells/total cells), yield or recovery (post-selection CD34 + cells/pre-selection CD34+ cells), and the level of depletion of total nucleated cells (TNC), B cells (CD19+ cells), and T cells (CD3+ cells) in the post-selected PBPC product as compared to the initial value (see Table 3 below). The median purity of CD34+ cells in the selected PBPC product was 90%, the median viability was 98%, and median recovery of CD34 cells was 51%. The performance was similar for the two device systems (300 and 300i).

Table 3 SUMMARY OF DEVICE PERFORMANCE						
	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

† # samples refers to number of Isolex procedures (a procedure involved one or, at most 2, leukapheresis products).

B. SUPPORTIVE STUDIES

In the major efficacy and supportive studies, it was noted that the assignment of day of engraftment could be subject to interpretation. Specifically, continuation or cessation of hematopoietic growth factors, on occasion, can influence the time to neutrophil engraftment, particularly early in engraftment. Similarly, the time to platelet recovery was generally recorded as the first day of a platelet count ≥ 20,000/μl even when that value occurred the day after the last platelet transfusion. In the review of the original PMA, FDA re-assessed time to engraftment. This resulted in designation of dates for time to engraftment that differed slightly from the assignment in the PMA. For platelet, the time to engraftment was arbitrarily assigned as the third day after the last platelet transfusion, in which all counts were sustained at ≥20,000/μl. This adjustment affected a limited number of subjects in both Isolex®-processed and control arms; the adjustment did not alter the outcome of the primary efficacy for the major efficacy study. In the description of the supportive studies, the information for time to engraftment includes both FDA's analysis based on re-assessment of time to engraftment for the information in the original PMA as well as an analysis based on the time to engraftment data provided by the Nexell (without adjustment by FDA) in the most recent update submitted to the PMA.

1. Protocol #92004-302104-A CD34+ Cells from Mobilized Peripheral Stem Cell Collections for Hematologic Rescue of Patients Treated with High-Dose Chemo/Radiotherapy for B-Cell Malignancies

Study Design

An open-label, single arm, multicenter study conducted in adults with advanced B-cell malignancies (predominantly non-Hodgkin s lymphoma and multiple myeloma). The Isolex® 300 device and the Isolex® 300i device were utilized sequentially during the course of the study.

Subjects had to meet eligibility requirements for institutional transplant protocols and achieve adequate mobilization (≥20 CD34+ cells/μl peripheral blood) following chemotherapy and G-CSF (5-10 μg/kg/d) or following G-CSF alone. A minimum of 6.5 x 10^6 CD34+ cells/kg were to be obtained during apheresis (5 x 10^6 CD34+ cells/kg for selection with the Isolex® device and 1.5 x 10^6 CD34+ cells/kg, unselected, to be cryopreserved as a back-up stem cell source). The transplantation procedure varied by center; two of the 12 participating centers used a tandem (two-transplant) approach. At one site, the stem cell product for the second transplant procedure underwent selection. At the other site, both PBPC products were selected, however only the data from the second transplant procedure were included in the analysis for this study.

Results

Patient disposition

A total of 234 patients were registered in the study. The disposition of patients is provided according to the device configuration in use during that portion of the study.

Isolex® 300

There were 157 patients enrolled in the trial during use of the Isolex 300 system. Of these, 62% (98) met eligibility for Isolex® -processing. Reasons for inability to continue on study/lack of eligibility for Isolex® processing were: failure to achieve adequate mobilization (n=42, 27% of total), failure to meet collection target of 5 x 10^6 CD34+ cells/kg (n=10, 6%), and failure to meet other eligibility criteria (n=7, 4%).

Among the 98 subjects who were eligible to undergo Isolex®-processing, 8 (8%) subjects had a total yield of less than 2 x10^6 CD34+ cells/kg in the selected product. In addition, 9 of the 10 subjects who failed to meet the target collection goal (i.e., had < 5 x 10^6 CD34+ cells/kg available prior to selection) underwent selection in violation of the protocol. Five of these nine subjects had a final yield of less than 2 x10^6 CD34+ cells/kg.

Isolex® 300i

A total of 77 patients were registered during the study during use of the Isolex 300i system. Of these 16 (21%) failed to achieve adequate mobilization, 7 (9%) failed to meet the target collection criterion ≥5 x 10^6 CD34+ cells/kg, and 4 (5%) patients were deemed ineligible to continue for other reasons. Fifty-seven patients underwent apheresis for further processing with a total of 50 patients (65% of those registered) meeting all criteria

for Isolex®-processing. There were 6 eligible subjects (12%) whose final yield was less than 2 x10^6 CD34+ cells/kg. Three of the 7 subjects who failed to meet the target collection goal also underwent selection; all three had a final yield of less than 2 x10^6 CD34+ cells/kg.

Mobilization failures
Based upon an analysis of the first 97 subjects enrolled, it was noted that patients who failed to achieve adequate mobilization were more likely to have received extensive chemotherapy (more than 3 prior chemotherapy regimens) than those who did mobilize (42% vs. 11%). The sponsor also performed an additional analysis in which three factors were identified as associated with a failure to achieve adequate mobilization. The three factors identified by the sponsor were: mobilization with G-CSF alone (as compared to chemotherapy plus G-CSF), diagnosis of non-Hodgkin's lymphoma, and number of prior chemotherapy regimens (<3 vs. ≥ 3 regimens). These exploratory analyses were retrospective and have not been confirmed by FDA.

Engraftment results
The results of time to engraftment were analyzed in detail by FDA reviewers for the first 71 patients enrolled who achieved the desired mobilization of CD34+ cells in the peripheral blood. The median CD34+ cell dose infused was 4.6 x 10^6 CD34+ cells/kg (0.9-23 x 10^6 cells/kg). The median time to neutrophil engraftment was 11 days (6-54 days) and median time to platelet engraftment was 13 days (range 6-84 days).

The sponsor's analysis of time to engraftment for 148 patients "evaluable for engraftment" provides similar results. A median dose of 4.5 x 10^6 CD34+ cells/kg was infused. The median time to neutrophil engraftment was 11 days (6-29) and the median time to platelet engraftment was 13 days (5-137 days).

Device performance
Data regarding device performance are summarized in Tables 5 and 6 .

2. Protocol 92004 302105 "Use of CD34+ Cells Isolated with the Baxter Isolex 300 System to Purify Peripheral Blood Progenitor Cells for Autotransplantation Following High-Dose Chemotherapy for Treatment of Advanced Stage, Low Grade non-Hodgkin's Lymphoma"

Design
An open-label, single arm, single center trial conducted in adults with advanced stage NHL or CLL, who had received moderate to no prior treatment. Subjects were to have adequate organ function, a Karnofsky performance status of >80%, and adequate mobilization of progenitor cells (≥20 CD34+ cells/μl peripheral blood) following Dexa-BEAM chemotherapy and G-CSF. In contrast to other protocols, patients also had to meet the following criteria in order to proceed to transplantation: evidence of clinical tumor response to induction/mobilization chemotherapy, low tumor volume (lesions <2 cm and <20% marrow infiltration) after the second cycle of DexaBEAM, and ≥ 2 x 10^6

CD34+ cells/kg after Isolex 300 selection. Patients meeting these criteria underwent a conditioning regimen of total body irradiation (2 Gy BID days -7, -6, -5) and cyclophosphamide 60 mg/kg on days -4 and -3 prior to PBPC infusion on day 0. Supportive care included G-CSF 10 μg/kg/day post-transplant.

Results

Patient disposition

A total of 29 patients were registered in the study. The disposition of patients is provided according to the device configuration in use during that portion of the study.

Isolex 300

There were 23 patients enrolled in the trial during use of the Isolex® 300 system. Of these, 2 (9%) failed to achieve adequate mobilization and an additional subject did not have clinical tumor response to the DexaBEAM induction regimen. There was no requirement for the minimum number of cells in the apheresis product as a condition for Isolex-processing; the remaining 20 patients (87%) underwent apheresis and Isolex® processing of the PBPC. In 8 of the 20 subjects (40%), the final yield was less than 2 $x10^6$ CD34+ cells/kg. One subject did not undergo transplantation due to enrichment of leukemic cells in the selected PBPC product.

Isolex 300i

There were 6 patients enrolled in the trial during use of the Isolex® 300i system. Of these, none failed to achieve adequate mobilization; one subject was removed from study for lack of tumor response to induction DexaBEAM therapy. The remaining 5 patients underwent apheresis with PBPC selection with the Isolex® 300i. All 5 subjects had a final yield of greater than 2 $x10^6$ CD34+ cells/kg.

Engraftment

The FDA performed a detailed review of the engraftment data for 8 of the first 18 subjects enrolled who underwent transplantation. A median of 3.1 x 10^6 CD34+ cells/kg was infused. Engraftment was rapid and uniform. The median time to engraftment for neutrophils was 9 days (range 9-10 days) and the median time to platelet engraftment was 12 days (range 10-19 days).

The sponsor has provided an analysis of 15 of the 25 subjects who underwent Isolex®-processing. This analysis excludes 4 of the 29 subjects enrolled, who were removed from study prior to apheresis (2 mobilization failures and 2 patients without a tumor response to DexaBEAM induction). In addition, the sponsor excluded one patient who did not undergo transplantation due to enrichment of leukemic cells in the selected product, eight patients with inadequate yield post-Isolex® processing (<2 x 10^6 CD34+ cells/kg) and one additional subject, described as an "apheresis failure". A median of 3.3 x 10^6 CD34+ cells/kg was infused in this selected subset. The median time to neutrophil engraftment was 9 days (7-10 days) and the median time to platelet engraftment was 11 days (10-17 days).

Device performance (including device failure)
Significant technical problems were observed during cell processing which resulted in inadequate CD34+ cell yield and removal of patients from the study. These problems included clumping during apheresis (n=1), clumping in the device (n=2), and enrichment of leukemic cells following selection observed in one subject with CLL.

Additional information regarding device performance is summarized in Tables 5 and 6.

3. Protocol #92004 301103 "Use of Isolated CD34+ cells from marrow of matched Related or Unrelated Donors for Allogeneic Bone Marrow Transplantation"

Design
An open-label, single arm trial conducted at two centers. Matched related and matched unrelated donors for patients with hematologic malignancies or myelodysplastic syndrome who met eligibility requirements for institutional transplant protocols were enrolled. The target cell collection for donors was $\geq 3 \times 10^8$ MNC/KG recipient weight.

Results
Patient disposition
Data from 15 patient/donor pairs were submitted in the PMA. Twelve of the donors were related and three were unrelated. The underlying disease for which transplantation was performed was CML (n=5), MDS (n=4), AML (n=3), ALL (n=2), and NHL (n=1).

Engraftment
The median collection was 3.1×10^{10} total MNC, with a range of $2.2\text{-}3.3 \times 10^{10}$; the adequacy of the cell collection (relative to the proposed target) cannot be assessed from the data provided in the application. The median dose of CD34+ cells infused in the matched, related transplants was 0.8×10^6 CD34+ cells/kg (range $0.3\text{-}2.9 \times 10^6$ CD34+ cells/kg). The median yield was 39% and the median purity was 60%. The median time to neutrophil engraftment was 10 days (8-11 days) and the median time to platelet engraftment was 16 days (11-41 days); all recipients of matched, related transplants engrafted. The range of CD34+ cells infused in the matched, unrelated transplant recipients was $1.3\text{-}3.9 \times 10^6$ CD34+ cells/kg. Two of the three recipients did not engraft; the third patient had neutrophil engraftment on day 28 and platelet engraftment on day 22.

Adverse events
There were five transplant-related deaths. These were the two patients with primary graft failure, pulmonary infection, CMV encephalitis, and EBV-lymphoma (the latter 3 deaths occurred in recipients of matched, related grafts). Although no acute GVHD was observed in the matched, related transplant recipients, three of the 11 patients surviving to day 100 developed extensive chronic GVHD.

4. Protocol #92004 302106 "A Pilot Study of CD34+ Peripheral Blood Stem Cell Transplantation in Patients with Advanced Malignancies Eligible for Allogeneic Transplantation"

Design

An open-label, single-arm, multicenter study. Data from 21 donor-recipient pairs were submitted to the PMA. The study was restricted to donors where the recipient was at high risk for acute GVHD, defined as recipients greater than age 40 with matched, related donors and those with matched, unrelated or mismatched, related donors. The recipients also needed to meet eligibility requirements for institutional protocols for allogeneic transplantation.

The donors underwent mobilization of progenitor cells with G-CSF 16 μg/kg/d, with initiation of apheresis on day 4, for a total of 4 aphereses or until a minimum of 7.5 x 10^6 CD34+ cells/kg had been collected. For the recipients, the myeloablative conditioning regimen and GVHD prophylaxis protocols varied by institution. One of the four participating sites did not administer G-CSF post-transplantation in all patients, restricting its use to patients with evidence of delayed engraftment or in those who received a low CD34+ cell dose.

Results

Patient disposition

Data on 21 donor-recipient pairs were provided. The transplant recipients had a variety of hematologic malignancies (AML n=5; CML n=4; MDS n=3; NHL n=3; myeloma n=2; ALL n=2; T-cell ALL n=1; CLL n=1). There were 16 matched, related and 5 mismatched, related PBPC products infused. The median number of CD34+ cells infused in recipients of matched related allografts was 5.3 x 10^6 CD34+ cells/kg (range 2.2-9.8 x 10^6 CD34+ cells/kg). The median number of CD34+ cells infused in all recipients was 5.2 x 10^6 CD34+ cells/kg. One subject received back-up cells at the time of selected cell infusion due to an inadequate CD34 cell dose in the selected product.

Engraftment

The median time to engraftment in recipients of matched, related PBPCT was 14 days for neutrophils (range 9-17 days) and 12 days for platelets (9-37 days). Engraftment was poorer in recipients of mismatched, related grafts. All 5 patients died before transplant day +50. Two of the 5 did not show evidence of neutrophil engraftment (one received back-up cells) and two did not show evidence of platelet engraftment prior to death; one of these subjects received back-up cells. Rapid neutrophil engraftment (day +10) was observed in the remaining mismatched, related recipient, who died of a fungal infection. A number of subjects died in the immediate peri-transplant and early transplant period and were not evaluable for assessment of acute or chronic GVHD. Three of 15 subjects developed severe (grade 3-4) acute GVHD and eight of eleven patients developed chronic GVHD. In three of the eight, this was limited and in five, this was extensive chronic GVHD. One subject developed an EBV lymphoma post-transplant.

Integrated Summary of Device Performance

Clinical Studies Experience (General applicability)

Across all clinical studies, there were a sizeable number of subjects enrolled who failed to successfully complete the trial. Specifically, patients were unable to complete the assigned treatment program and receive Isolex®-processed cells for the following reasons:

1) failed to achieve successful mobilization (≥ 20 CD34+ cells/μl peripheral blood),
2) failed to meet target collection requirements, i.e., to collect sufficient cells for further processing (≥ 6.5 x 10^6 CD34+ cells/kg to obtain ≥ 5 x 10^6 CD34+ cells/kg for selection), despite evidence of adequate mobilization, and
3) failure to obtain adequate numbers of CD34+ cells (<2 x 10^6 CD34+ cells/kg) in the selected product to ensure rapid engraftment, despite an adequate CD34+ cell count in the initial (pre-selected) PBPC product. The patient disposition for the clinical studies is summarized in the table below.

Table 4
Disposition of Patients in Autologous Transplantation Trials
According to Reasons for Study Discontinuation

Study, Device	Total patients enrolled	Not eligible for Processing[1,2]		Subjects eligible/ randomized to Processed arm	Transplanted In Processed Arm	Target Collection Failures[4]	Yield <2 x 10^6 CD34 cells/kg
		Mobil. Failure[3]	Other				
302103, 300	75	14	11	59/28	28	5	10
302103, 300I	114	17	5	92/48	47	4	7
302104-A, 300	157	42	7	108/108	105	10	13
302104-A, 300i	77	16	4	57/57	55	7	6
302105, 300	23	2	1	20/20	20	NA[4]	8
302105, 300I	6	0	1	5/ 5	5	NA[4]	0

1. Patients were not randomized (302103) or not eligible (302104-A) due to failure to meet mobilization criteria (≥ 20CD34/μL) or for failure to meet other inclusion/exclusion criteria ("other").
2. For protocol 302105, eligibility was in two stages (i) successful mobilization (≥ 20CD34/μL), apheresis and selection, with target of ≥ 2 x 10^6 CD34 cells/kg after selection and (ii) dexaBEAM cycle 2 – patients require complete or very good partial response to continue on study.
3. Mobilization failures defined as failure to reach ≥ 20 CD34 cells/μL in peripheral blood during mobilization.
4. For protocols 302103 and 302104-A, target collection was defined as collection of ≥ 5 x 10^6 CD34 cells/kg available for selection; patients with < 5 x 10^6 CD34 cells/kg were not permitted to undergo Isolex-selection. There was no pre-selection target in protocol 302015.

Across all studies performed of autologous PBPC transplantation, the proportion of subjects registered who were able to undergo randomization and/or Isolex® processing ranged from 69% to 87%. The majority of these subjects did not undergo Isolex® processing due to failure to achieve adequate mobilization. Of those who remained on study, 12-40% with a PBPC product processed with the Isolex® 300 device and 0-15% with a PBPC product which was processed with the Isolex® 300i device had a final CD34+ cell yield of < 2 x 10^6 CD34+ cells/kg.

The differences observed in the rates of patients achieving the desired CD34+ cell target among the various clinical studies might be a reflection of the rigor in patient selection and degree of adherence to the protocol. It should be noted that the Isolex® 300i system was introduced at a

later time into ongoing studies, when investigators were more experienced with both the device and the protocols. In the latter half of the protocols, concurrent with the introduction of the Isolex® 300i system, the rate of patients failing to achieve mobilization targets also decreased. In the major efficacy study (302103), better a higher mobilization rate may have been related to patient selection, in that the subjects enrolled had less advanced disease and were less heavily pretreated that those enrolled in the earlier portion of the study. While experience and other factors may account for the lower dropout/ineligibility rate observed during use of the Isolex® 300i system, one cannot exclude the possibility that the Isolex® 300 system is less efficient in cell yield and recovery. Direct comparisons of the two systems should be conducted in appropriately designed clinical trials to resolve this issue.

Device Performance:
Device performance was assessed by measurement of the reduction in non-target (CD34 negative) cells, and the purity, viability and recovery (yield) of CD34+ cells in the Isolex®-selected product. Device performance varied by source of progenitor cells; there appeared to be less efficient enrichment of CD34+ cells observed with bone marrow as compared to peripheral blood progenitor cells. In studies using peripheral blood progenitor cells, there was a reduction in the total nucleated cells (TNC) by approximately 2-3 logs. The median purity of CD34+ cells in the selected products was 90%, the median viability was >92%, and the median recovery of CD34+ cells from the starting apheresis products was 25-51% (depending on study and device).

TABLE 5
Integrated Summary of Isolex® 300i Device Performance Across Studies

Study	Median Purity of CD34+ cells	Median CD34 cell Recovery	Median log reduction in MNC	Median log reduction in CD3+ cells	Median log reduction in CD19+ cells
Pivotal 302103	90% (9-99) n=82	54% (3-100) n=82	>2	3.4 (1.7-4.3) n=54	2.0 (0.2-3.6) n=20
Supportive studies in PBPC					
302104-A	91% (11-99) n=108	51% (3-100) n=108	>2	3.4 (1.8-4.4) n=50	2.4 (0.9-3.8) n=48
302105	99% (93-99) n=8	48% (11-86) n=8		2.9 (1.6-3.6) n=5	N/A

"n" refers to number of Isolex procedures, which involve 1-2 mobilized apheresis products for PBPC

TABLE 6
Integrated Summary of Isolex® 300 Device Performance Across Studies

Study	Median Purity of CD34+ cells	Median CD34 cell Recovery	Median log reduction in MNC	Median log reduction in CD3+ cells	Median log reduction in CD19+ cells
Pivotal 302103	90% (55-99) n=50	45%(16-100) n=50	>2	3.5 (2.2-4.7) n=30	2.3 (1.0-3.0) n=21
Supportive studies in PBPCT					
302104-A	90% (11-99) n=209	42% (1-100) n=206	>2	3.2 (1.5-4.8) n=93	2.9 (1.6-4.5) n=52
302105	88% (3-99) n=34	24% (0-65) n=34	>2	3.3 (2.1-4.8) n=22	2.4 (0.6-3.6) n=8
302106 (n=21) [normal donors]	92%	43%	>2	3.8	4.2
Supportive studies in BMT					
301103 (n =15)	60%	39%	>2	2.6	2.2

"n" refers to the number of Isolex procedures

Tumor depletion studies:
Depletion of breast tumor cells was assessed in a semi-quantitative manner 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.

Engraftment Results
In the pivotal study, study data were provided for the intent-to-treat population, i.e., for all patients who received a PBPC transplant. In this study, the median times to neutrophil and platelet engraftment were statistically significantly longer for those randomized to receive Isolex®-processed cells compared to those randomized to unmanipulated PBPC, based on log-rank test of the Kaplan-Meier curves. However, the differences were not deemed to be clinically important and the study excluded a median prolongation in time to neutrophil engraftment of more than 3 days. In the two single arm, supportive studies, based upon data obtained from the majority of subjects who underwent transplantation, the median time to neutrophil and platelet engraftment is similar to that observed in Isolex®-processed arm of the randomized efficacy

study. In all three studies, there was evidence that a small group of patients had delays in neutrophil and platelet engraftment which may have had clinical consequences, however none of the studies were designed to capture the clinical consequences of these delays or impact on the quality of life. The delay in time to platelet engraftment was greater in duration and observed more frequently than delay in time to neutrophil engraftment.

Use of "back-up" progenitor cell products was documented in two of the three studies. Back-up PBPC products were administered for documented engraftment delays and, in some instances, concern on the part of the investigators that the number of cells to be infused might result in engraftment delay. Back-up PBPC products were administered in approximately 10% of the patients who were to receive Isolex®-processed PBPC products alone. The sponsor has not identified any patient with secondary graft failure, however there have been a limited number of patients who required long-term support for marginal engraftment (e.g., platelet and red blood cell transfusions, G-CSF, second PBPC infusion). Due to the poor quality of the long-term follow-up information, it is difficult to determine the extent to which the use of Isolex®-selected PBPC alone is responsible versus the relative contribution of other factors, such as relapse, and concomitant medications, particularly cytotoxic therapies.

X. CONCLUSIONS DRAWN FROM THE CLINICAL STUDIES

Risk/Benefit Analysis

The clinical benefit of positive selection is, at this time, theoretical and is based on the hypothesis that removal of tumor cells prior to infusion of an autologous transplant product will improve the disease-free survival of patients for whom the high-dose, myeloablative chemotherapy may be curative. Neither the major efficacy trial for the Isolex® System, nor the major efficacy trial for a related product (CEPRATE® SC System) have shown a significant difference in progression-free survival or overall survival for the positive-selection (tumor depletion) arm. The additional risks of infusion of Isolex®-selected PBPC products containing at least 2 x 10^6 CD34 cells/kg, as compared to unselected PBPC are minor, particularly in light of the toxicity of the overall procedure. These additional risks are limited to clinically insignificant delays in time to engraftment and small increases in the RBC transfusion requirements for the majority of the patients and clinically significant delays in engraftment in a small number of patients. Given the serious potential complications of high-dose chemotherapy requiring autologous progenitor cells for hematopoietic reconstitution (including up to a 5% mortality rate), the additional risks incurred with the use of Isolex®-selection are small, acceptable to patients and physicians, and balanced by the potential for benefit.

Safety

The clinical engraftment data demonstrate that there is no clinically significant delay in the time to neutrophil or platelet engraftment when patients are transplanted with Isolex®-selected PBPC products following myeloablative therapy as compared to patients transplanted with unselected PBPC. There was however, a statistically significant delay in the time to engraftment for both neutrophils and platelets (log-rank test) for patients who received Isolex-selected PBPC as compared to control. The 95% confidence intervals indicate that the maximum potential

difference between these groups is 1 day for neutrophil engraftment and 3 days for platelet engraftment. At a median dose of greater than 2 x 10^6 CD34 cells/kg, Isolex® -selected transplant products produced rapid and stable engraftment.

The applicability of this procedure to the general population has not been determined. In the major efficacy trial, 16% of patients who were registered were not randomized due to inadequate evidence of PBPC mobilization (>20 CD34+ cells/μl); thus there are no data on the safety of this processing procedure in patients who are difficult to mobilize. In addition, there were also 22% of patients with evidence of adequate PBPC mobilization, in whom <2 x 10^6 CD34+ cells/kg were available post-Isolex® processing. Factors that are associated with poor yield post-processing have not been identified. Based on the yield of CD34+ cells post-Isolex® processing, one should assume up to 60% reduction in the absolute CD34+ cell number in the selected product. In patients who received <2 x 10^6 CD34+ cells/kg, there were further delays in the time to engraftment and use of the device in patients who are likely to have a final yield of <2 x 10^6 CD34+ cells/kg is not recommended.

In the controlled clinical trial, there were no statistically significant differences in the incidence of infections, use of antibiotics or length of hospital stays. There was very little infusional toxicity related to any cell infusion products; most toxicities were due to the chemotherapy and other medications, and were only temporally associated with cell infusion. There was a statistically significant increase in the requirement for RBC transfusions for patients in the Isolex® arm (median 5.2 units RBC/patient vs. 4.4 units RBC/patient [Isolex®/unselected]).

Aside from the potential for delay in time to engraftment, which may in a small number of patients be clinically important, use of this device carries the potential risk of introduction of pathogenic contaminants as a result of additional processing. While no clinically significant infections have been identified as a result of such contamination, culture of the PBPC product has identified pathogenic contaminants.

Efficacy

Processing of mobilized peripheral blood progenitor cells, collected in several disease settings (breast cancer, non-Hodgkin's lymphoma, multiple myeloma, and chronic lymphocytic leukemia) results in a non-specific loss of CD34 negative cells including tumor cells, with a 2-3 fold reduction in malignant cells contaminating clinical apheresis samples. The clinical relevance of this reduction is not known as there has been no demonstration of any impact on overall or progression-free survival in patients who receive processed PBPC. However, there is a theoretical benefit to removal of tumor cells contaminating PBPC products as it has been shown in certain pediatric tumors that tumor cells in the infusate can contribute to relapse.

XI. PANEL RECOMMENDATION

In 1994, CBER sought and received advice from members of the Biologics Response Modifiers Advisory Committee (BRMAC) regarding development of hematopoietic support therapies. The following guidance and advice were provided to CBER regarding study design and standards for approval for products, including devices, intended for tumor cell and/or T-cell depletion of stem

cell products used for hematopoietic reconstitution. The following advice was provided which is relevant to this PMA:

- The potential to adversely affect the graft exists any time there is manipulation of progenitor cells; however, for most of the modalities, late graft failure has not been widely observed. The greatest risk for late failures is transplantation in the allogeneic setting following TCD.
- For purging agents, a substantial reduction in tumor cells in the graft could be the basis for an approval, provided there were no detrimental effects on engraftment (as demonstrated through randomized trials).
- The tumor types most readily amenable to testing are ones where sensitive markers exist: non-Hodgkin's lymphoma, multiple myeloma, and breast cancer.

On July 24, 1997, the PMA 97-0001 was presented and discussed before the BRMAC. The data in the application was limited to PBPC processing with the Isolex® 300 device (not the 300i device). The safety and efficacy data consisted of results from 47 subjects who were randomized in Protocol 302103 (the major efficacy study), of whom 26 underwent Isolex 300-selection and 21 were randomized to the unprocessed arm. No data were provided regarding tumor reduction in the Isolex® 300-processed PBPC products.

The Committee voted on the following questions:

1. Are these data adequate to establish that, in patients with breast cancer who undergo PBPC transplantation, Isolex® processing does not substantially impair the engraftability of a cell population, i.e., that it yields a cell population effective for transplantation and engraftment?

   BRMAC vote and comments: Committee discussion centered around the small sample size of the study and the need to separate the investigator's perception of device potential from clinical benefit. The Committee voted 13 yes, 2 no, with 1 abstention that the data from breast cancer patients give adequate information that the device yields a cell population that is effective for transplantation and engraftment.

2. In the case of this CD34+ cell selection device for autologous PBPC transplantation, should failure to impair engraftment substantially *per se* be considered evidence of efficacy?

   Following a discussion by the Committee regarding the definition of efficacy and whether or not the data support clinical benefit to the patient, the Committee voted 6 yes, 5 no, with 5 abstentions, on whether failure to impair engraftment be considered evidence of efficacy.

   In additional discussion (with no votes), there was a general sense by the Committee that where there is the implication that a device works by purging tumor cells, data would need to show a depletion of tumor cells and demonstrate engraftment efficacy.

XII. FDA DECISION

CBER concurred with the BRMAC's recommendation of July 24, 1997. After review of additional information that was submitted in major amendments on July 1 and July 30, 1997, FDA issued a letter to Nexell Therapeutics, on November 21, 1997, advising that its PMA was not approvable subject to submission of information demonstrating that the Isolex® 300 device was capable of reducing a clinically significant amount of tumor contaminating the mobilized PBPC product. The information that was requested was available primarily with a different configuration (the Isolex® 300i) for which clinical safety information and performance data had not been supplied. Nexell Therapeutics supplied the required data as follows:

1. December 17, 1997: tumor purging data for Isolex® 300 and 300i
2. February 2, 1998: submission of clinical study and device performance data for the Isolex® 300i, including tumor purging data.
3. April 24, and June 9, 1998: Partial SAS data set for Isolex® 300i clinical data
4. August 27, 1998: Response to FDA letter of July 2, 1998 and SAS-dataset for the Isolex® 300i with additional clinical data, addressing missing data, and updated follow-up.
5. December 1, 1998: SAS-dataset integrating clinical data and performance data for Isolex® 300 and Isolex® 300i devices; updated information regarding time to engraftment, overall survival and time-to-progression for major efficacy trial.
6. February 9,1999: Response to FDA letter of January 7, 1999.

FDA issued an approval order on July 2, 1999. The sponsor's manufacturing facilities and certain contract manufacturing facilities were inspected on 2/8/99-2/18/99 and 3/25/99; 2/11/99; 2/18/99; 2/15-2/25/99; 2/16-3/1/99; and 3/1-3/3/99, and were found to be in compliance with the device Good Manufacturing Practice regulations.

XIII. APPROVAL SPECIFICATIONS

Instructions for use: See labeling (Attachment 1)

Hazards to Health from Use of the Device: See Indications, Contraindications, Warnings, Precautions, and Adverse Events in the labeling (Attachment 1)

Post-approval Requirements and Restrictions: See approval order (Attachment 2).