Published in final edited form as:

Cytotherapy. 2010 April ; 12(2): 170–177. doi:10.3109/14653240903476446.

CD34⁺ Cell Selection Using Small Volume Marrow Aspirates: A Platform for Novel Cell Therapies and Regenerative Medicine

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Abstract

Background—The study was initiated to determine if CD34⁺ cell selection of small volume bone marrow samples could be effectively performed on the Isolex® 300i Magnetic Cell Selection System® device and if the results obtained from these samples would be comparable to results from large standard volume samples. The impact on the CD34⁺ recovery using a full versus a half vial of Isolex® CD34 reagent and the effects of shipping a post-selection product were evaluated.

Study Design and Methods—A protocol to evaluate CD34⁺ cell selection with two ranges of smaller volume bone marrow samples (~50 mL and ~100mL) was developed and instituted at the three PACT facilities. The study was performed in 2 phases.

Phase I—Mean post-selection CD34⁺ recoveries from the two sizes of samples were (104.1% and 103.3% (smallest and largest volumes, respectively), and mean CD34⁺ recoveries were 115.6% and 88.7% with full and half vials of reagent respectively. Mean CD34⁺ recoveries for post-shipment smaller volume samples (106.8%) and for larger volume samples (116.4%) and mean CD34⁺ recoveries of 99.9% and 127.4% for post-shipment samples processed with full and half vials of reagent respectively were obtained.

Phase II—Mean CD34⁺ recovery was 76.8% for post-selection samples and 74.0% for post-shipment samples.

Discussion—Results from this study suggest that smaller volume bone marrow sample processing on the Isolex® system is as efficient or more efficient when compared to standard volume sample processing. Post-processing mean CD34⁺ recovery results obtained using a full vial or a half vial of CD34 reagent were not significantly different.

Keywords

CD34⁺ cell selection; bone marrow; cell therapy; regenerative medicine

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Introduction

Bone marrow, the original source of hematopoietic stem cells (HSCs) for transplantation [1], is increasingly becoming a source of various cell types for a number of diverse, clinical applications. Mesenchymal stromal cells (MSCs), traditionally isolated and expanded from bone marrow, have been used for treatment of several diseases, including graft-versus-host disease [2] and inflammatory bowel disease [3], among others [4]. Likewise, marrow-derived mononuclear cells (MNCs) have been used for a variety of applications, including cardiac [5] and peripheral vascular disease [6] and as part of a cell therapy-based approach to repair of spinal cord injury [7]. Finally, specific subsets of cells (e.g., CD34⁺ cells, CD133⁺ cells) isolated by immunomagnetic selection have been used for similar purposes such as treatment of cardiovascular disease [8]. Many other trials are currently underway or in various stages of development. Most of these novel regenerative medicine applications involve aspiration of marrow volumes of much less than that harvested for “standard-of-care” hematopoietic stem cell transplantation. Often volumes as small as 25–100 mL are collected under local anesthesia; in the context of certain types of cell processing techniques or technologies, small volume samples may provide a challenge to cell processing facilities. As an example, clinical-scale immunomagnetic cell selection devices have minimum volume loading requirements and may have minimum or ideal target cell counts. Further, these devices were originally designed for apheresis collections, and, as such, red cell contamination with bone marrow may hinder their function.

In an effort to further delineate the approach to smaller volume marrow processing on a clinical-scale immunomagnetic cell selection device, we initiated a study with the Isolex® 300i Magnetic Cell Selection System® (Baxter, Deerfield, Illinois), a CD34⁺ cell selection device. In addition to defining the effect of smaller volume marrow on instrument efficacy, the impact of the amount of Isolex® CD34 reagent (full versus half vial) and shipping of a post-Isolex® selected product were studied. This study was not designed to evaluate inter-laboratory or intra-laboratory variability.

This project was performed by Production Assistance for Cellular Therapies, or PACT, an NHLBI-sponsored program that consists of the Center for Cell and Gene Therapy of the Baylor College of Medicine, Molecular & Cellular Therapeutics of the University of Minnesota, the University of Pittsburgh Cancer Institute, and the EMMES Corporation, the administrative center. The primary charge of the PACT group is to provide translational support and clinical production of cell therapy products for institutions engaged in clinical research involving cellular therapies and regenerative medicine. The feasibility of regional cell processing and maintaining satisfactory shipping conditions is critical to achieving these goals.

Methods

Phase I - University of Minnesota

Phase I involved processing of different small volumes of bone marrow [“smaller”, ~50 mL (n=4) versus “larger”, ~100 mL (n=5)], using a full vial or half vial of Isolex® (CD34) Reagent Kit, and shipping to Columbus, Ohio and back to Minnesota (AirNet Systems Inc., Columbus, Ohio). Analysis included measurement of initial volume, total nucleated cell count, CD34⁺ cell enumeration, viability, CFU-GM, and sterility testing from the initial, post-Isolex® selection, and post-shipment products.

Phase II - Baylor College of Medicine and University of Pittsburgh

Phase II further evaluated processing of additional bone marrow samples [~100 mL (n=4 at each site)] with a full vial of Isolex® (CD34) reagent and included shipping to Columbus, OH

and back to the processing site for post-shipment analysis. Testing included measurement of initial volume, total nucleated cell count, CD34⁺ cell enumeration, and viability from the initial, post-Isolex® selection, and post-shipment products. Repeat analysis of flow cytometry data files at one site (University of Pittsburgh) was included as an additional exercise relating to standardization of CD34⁺ cell enumeration.

Cell Processing

Sample preparation

Fresh, unfiltered bone marrow samples from healthy donors were received from Lonza Inc. [Walkersville, Maryland] in 50 mL conical tubes with sodium heparin. Cell processing was based upon the manufacturer's standard procedure (Isolex® version 2.5) with modification. The fresh marrow samples were diluted to 500 mL with working buffer solution [PBS, 4% sodium citrate, 25% human serum albumin (HSA)]. There was no prior processing such as MNC separation on the COBE Spectra or red cell removal with hydroxyethyl starch (HES). ACD-A (10%) was not added to the fresh marrow samples.

CD34⁺ cell selection

Briefly, the Isolex® utilizes an unconjugated murine monoclonal anti-CD34 antibody (9C5) as the primary antibody. After the antibody is allowed to coat the CD34⁺ cells and following a washing step to remove unbound antibody, 4.5 micron magnetic beads (Dynabeads® M-450) coated with polyclonal sheep anti-mouse immunoglobulin are added. Bead-target cell rosette complexes form and CD34⁺ cells are then captured by an open field magnetic system. Following a washing step in the selection chamber, a PR34⁺ stem cell releasing agent is added. The beads and associated antibodies are retained while the cells are released, washed, and collected.

Post- Isolex® selection and shipping

Post-processed products were diluted with working buffer solution to provide adequate volume for shipping studies for both Phase I and Phase II. Products were shipped in insulated shipping containers (cardboard box with Styrofoam insert) surrounded by refrigerated stabilizing packs, and a continuous temperature monitoring device (ShipsLog, Chart Industries, Inc., Marietta, GA) was placed in each container. Marrow samples were accepted at the receiving laboratory within 14 hours (mean) of initiation of shipment for Phase I and 19 hours (mean) for Phase II.

Quality Control Testing

Total Nucleated Cell Count

Samples were analyzed according to standard operating procedures on the Coulter®Ac.T diff™ (Beckman Coulter, Inc., Fullerton, CA) at Minnesota and Pittsburgh and the Coulter®AcT8 analyzer (Beckman Coulter, Inc., Fullerton, CA) at Baylor.

Flow Cytometry

CD34⁺ cell enumeration was performed according to ISHAGE guidelines [9]. Minnesota and Baylor employed a dual platform analysis. Samples were incubated with fluorochrome-conjugated anti-CD34⁺ cell monoclonal antibody, fluorochrome-conjugated anti-CD45⁺ monoclonal antibody (BD Biosciences, Franklin Lakes, NJ), and 7-amino-actinomycin D (7-AAD, Via-Probe™, BD Biosciences, Franklin Lakes, NJ). A minimum of 100,000 events were collected with the FACSCalibur (BD Biosciences, Franklin Lakes, NJ) at Minnesota and the FACSCanto-II (BD Biosciences) at Baylor.

Pittsburgh employed a single platform approach to analysis on the Beckman Coulter Epics XL [Beckman-Coulter, Fullerton, CA.]. Samples were incubated with fluorochrome-conjugated anti-CD34⁺ cell monoclonal antibody, fluorochrome-conjugated anti-CD45 monoclonal antibody [Beckman-Coulter, Fullerton, CA.] and 7-amino-actinomycin D [7-AAD, (Beckman-Coulter, Fullerton, CA.)], and a minimum number of 75,000 events were collected.

Flow cytometry analysis included additional markers: CD3, CD19, and CD133 (2 of 3 centers). Centers employed institutional standard procedures for analysis of these markers.

Colony - Forming Unit - Granulocyte Macrophage (CFU-GM)

A 0.2 mL sample of marrow was diluted in an in-house progenitor media containing 2% fetal bovine serum (FBS), 5mL L-Glutamine, 10mL Pen-Strep to a concentration of 3.0×10^6 NC/mL for fresh samples and $0.5\text{--}1.0 \times 10^6$ NC/mL for post-Isolex® selection and post-shipments samples. The sample was then diluted 1:10 in MethoCult™ GF H4534 “Complete” Methylcellulose Media containing recombinant cytokines without erythropoietin (StemCell Technologies, Vancouver, British Columbia, Canada). The final concentration was determined and 1.0 mL of the suspension (containing 20,000–40,000 cells) was plated in duplicate on a 12 well tissue culture plate and incubated at 37°C, 5% CO₂ in 95% humidity chamber for 15 days. CFU-GM colonies containing > 40 cells were scored using a dissecting microscope. CFU-GM colonies were enumerated and averaged between the two wells.

Sterility

A 2.0 mL cell aliquot was removed from the product for sterility testing on the BACTEC System (BD Biosciences, Franklin Lakes, NJ). Aerobic and anaerobic cultures were performed and held for a total of 14 days before being considered as negative.

Results

Tables 1a–c summarize the results of the initial studies (Phase I) conducted at the University of Minnesota. Bone marrow samples averaged 58.1 mL (n=4, range 51.0 – 62.4 mL) for the small volume and 105.9 mL (n=5, range 88.8 – 120 mL) for the large volume. Mean cell concentration was 2.36×10^7 cells/mL for the small volume samples and 2.79×10^7 cells/mL for the larger volume samples. Mean CD34⁺ cell recovery for Phase I post-Isolex® selection samples was 104.1% for smaller volume samples and 103.3% for larger volume samples. Mean CD34⁺ cell recovery for Phase I post-shipment samples was 106.8% for smaller volume samples and 116.4% for larger volume samples. Viability results (7-AAD) were excellent at all stages of sample processing with a mean of 98.1% (initial), 98.5% (post-Isolex® selection), and 97.7% (post-shipping). Pre-processing CFU-GM averaged 1192 colonies/ 10^6 cells plated; post-Isolex® selection and post-shipping CFU-GM averages were excellent as expected, greater than 5635 colonies/ 10^6 cells plated and greater than 4255 colonies/ 10^6 cells plated, respectively. Sterility testing for all bone marrow samples was negative.

The post-Isolex® selection mean CD34⁺ cell recovery was 115.6% (range 88.7% – 135.6%) for samples processed with a full vial of reagent and 88.7% (range 43.9% – 116.1%) for samples processed with a half vial of reagent. The post-shipment mean CD34⁺ cell recovery was 99.9% (range 40.2% – 205.9%) for samples processed with a full vial of reagent and 127.4% (range 76.4% – 210.4%) for samples processed with a half vial of reagent. Because of the small number of samples tested, no difference in the average CD34⁺ cell recovery percentage could be detected between the full versus half vial samples.

Results of the follow-up studies performed at Baylor and Pittsburgh (Phase II) using larger volume marrow samples (n=8, mean volume of 109.7 mL) with a full vial of Isolex® CD34

reagent are shown in Table 2a. Mean cell concentration for all 8 different samples analyzed was 2.21×10^7 cells/mL. Mean CD34⁺ cell recovery was 76.8% (Range 39.4 – 92.8 %) post-Isolex® selection and 74.0% (Range 43.3 – 96.1 %) post-shipment. Viability (7-AAD) was maintained at all stages (i.e., initial, post-Isolex® selection, and post-shipping) with means of 95.4%, 85.5% and 91.1% respectively (Phase II viability data not shown).

Figure 1 shows a typical flow cytometry analysis in this study. Figure 2 further describes the immunophenotyping results obtained for the bone marrow samples during the different stages of cell processing. The mean percentages for all flow markers tested are displayed in this figure.

An exercise in standardization was conducted at the University of Pittsburgh to reanalyze their flow cytometry data files from the Phase II portion of this study (Table 2b – Samples 15–18). Standardization of the gating strategy showed relative consistency in analysis with the unmanipulated marrow samples in regards to the CD34⁺ percentage and absolute CD34⁺ cell count. The mean CD34⁺ percentage and mean absolute CD34⁺ cell counts for the original analysis performed at the University of Pittsburgh were 0.51% and 9.04×10^6 cells/mL and the results from the reanalysis were 0.51% and 9.22×10^6 cells/mL respectively.

The mean CD34⁺ percentage, mean absolute CD34⁺ cell count, and the mean CD34⁺ recovery for the original analysis of the post-Isolex® selection samples performed at University of Pittsburgh were 59.8%, 7.25×10^6 cells/mL, and 81.7% and the results from the reanalysis were 59.4%, 7.58×10^6 cells/mL, and 83.8% respectively, which again showed consistency in analysis.

The mean CD34⁺ percentage, mean absolute CD34⁺ cell count, and the mean CD34⁺ recovery for the original analysis of the post-shipment samples were 60.8%, 2.28×10^6 cells/mL, and 61.6% and the results from the reanalysis were 60.7%, 9.88×10^6 cells/mL, and 129.7% respectively. One CD34⁺ recovery outlier (#18 = 227%) was observed in the post-shipment reanalysis. If this sample were removed from analysis, the original analysis of the post-shipment results would be 57.6%, 2.20×10^6 cells/mL, and 57.2% respectively and the post-shipment results upon reanalysis would be 58.2%, 7.39×10^6 cells/mL, and 97.2% respectively.

Discussion

As bone marrow becomes a more utilized source of cells for novel cell therapies and regenerative medicine, approaches to cell processing may require modification to accommodate smaller volume samples. The need for modification may be most obvious for immunomagnetic selection of specific subpopulations of cells. Immunomagnetic selection devices were designed for processing apheresis products and higher hematocrit values have been shown to be a hindrance to their function [10,11]. A minimum volume of product is required as well; while this requirement may be addressed by simply diluting the starting material with a buffer solution, dilution would not address the possible need for a minimum or optimal target cell count.

The manufacturer recommends that a maximum of 8×10^{10} nucleated cells be loaded onto the instrument. There is no maximum or minimum target (CD34⁺) cell number recommendation. However, as with all antigen-antibody reactions, there is an optimal relative antigen: antibody concentration. The optimal proportion of antigen: antibody would allow for the most efficient interaction, and, hence, cell selection. The Isolex® Anti-CD34 Monoclonal Antibody vials contain 2.5 mL (1.0 mg/mL) in sterile, non-pyrogenic phosphate buffered saline solution, and the Dynabeads® are packaged in vials containing 4×10^9 beads suspended in 10 mL of phosphate buffered saline with 0.1% human serum albumin.

The Isolex® Stem Cell Reagent Kit is optimized for use with larger mononuclear cell apheresis collections. We suspected that selections using smaller volume marrow samples would not be as efficient, and we initiated this study to further define the efficiency of such selections. Our results suggest that smaller volume marrow processing is as efficient or more efficient when compared to the processing of larger volume products.

Several investigators have shown consistency in mean CD34⁺ cell recovery with mobilized peripheral blood apheresis collections and the Isolex® system with Hildebrandt et al. [10] reporting 53.6%, Rowley et al. [12] reporting 58.4%, and Gryn et al. [13] reporting 55%. Final product purity in these three studies was consistent as well at 85.36%, 90.8%, and 91.7%, respectively [10,12,13]. However, CD34⁺ cell selection using the Isolex® and larger volume marrow has been shown to be less efficient when compared to mobilized peripheral blood [11]. Kasow et al. reported 29.93% recovery and 92.02% purity with primary bone marrow grafts (n=20). They hypothesized that greater hematocrit/red blood cell volume in the marrow collections led to these less efficient results. To minimize the negative effect of red cells on selection efficiency, Kasow et al. [11] performed an inverted centrifugation step (400 g for 10 minutes) and attained a red cell mean volume of 21.5 mL (range: 12.0 – 28.1 mL). We, however, did not attempt to remove the red cells prior to selection and initiated processing with a mean volume of 34.5 mL (range: 16.7 – 45.0 mL).

Because the Isolex® reagents are packaged for optimal selection of larger products, we included a comparison of full vial reagent versus half vial reagent in Phase I of the study. Four CD34⁺ cell selections were performed with half of the typical amount of Isolex® Anti-CD34 Monoclonal Antibody and half of the Dynabeads®. Qualitatively, there was no significant difference noted with the post- Isolex® selection mean CD34⁺ cell recovery being 115.6% and 88.7% for full vial and half vial, respectively. The post- Isolex® selection purity results were almost identical with mean values of 84.7% and 82.8% for full vial and half vial, respectively. We suspect this lack of difference was due to antibody/bead saturation with smaller volume marrows even when only a half vial was used during processing. Use of lesser amounts of reagents may be an option if the manufacturer is willing to consider alternatives to current packaging of kits.

The final products in this small study had relatively few cells, and this raises a few technical considerations. Of course, optimal cell doses for most (if not all) regenerative medicine/cellular therapies are not known, and, perhaps, few cells are needed for certain clinical applications. However, this study emphasizes the need for high quality starting material. The largest final doses were generally obtained from bone marrow samples with the highest total nucleated cell counts. Successful marrow aspiration is dependent upon technique and several small aspirations following re-positioning of the aspirate needle are necessary. Finally, when dose is limited, final QC testing should be performed on as few cells as possible or even the negative fraction (i.e., when technically feasible and if the FDA agrees with the plan).

In this study, CD34⁺ cell recoveries were widely variable and occasionally greater than 100%. The variability is likely to be at least in part, attributable to the small 'n' of the study. Recoveries greater than 100% have been reported previously with hematopoietic stem cells [14], and we believe that the initial results (i.e., pre-selection) for CD34%/count may have been underestimations of "rare" events. An under-estimate at that stage would cause the perception of >100% recovery with presumably more accurate analysis of the post-processing/post-shipping samples. Likewise, the lower overall recoveries in phase II may have been secondary to overestimation of "rare" events with the starting material. Certainly a larger study with a focus on intra-and inter-laboratory standardization of flow cytometry would be a logical next step.

Inter-laboratory standardization of quality control testing, including flow cytometry, was intentionally not considered in this exercise. Despite the inherent differences among each of the individual samples, reanalysis of Phase II data at Pittsburgh showed consistency in evaluation of the unmanipulated samples. Other studies have shown that standardization of CD34⁺ cell enumeration is possible if attention is paid to the nuances of flow cytometry gating strategy [15].

In summary, the primary charge of the PACT group is to provide translational support and clinical production of cell therapy products for institutions engaged in clinical research involving cellular therapies and regenerative medicine. The success of this study demonstrates the feasibility of regional cell processing of one such product. Smaller volume samples of bone marrow were efficiently processed using an immunomagnetic selection device designed for apheresis products. Further, satisfactory shipping conditions were maintained throughout each shipment, and stability of the final product was shown by good post-shipping cell recovery, viability, CFU-GM, and negative sterility testing.

Acknowledgments

This project was supported by NHLBI contract #s N01-HB-37163, N01-HB-37164, N01-HB-37165, and N01-HB-37166 from the National Heart Lung and Blood Institute.

This work was supported in part by PACT (NHLBI contract #s N01-HB-37163, N01-HB-37164, N01-HB-37165, and N01-HB-37166.) The authors would like to thank the staff at Baylor College of Medicine, University of Minnesota, and the University of Pittsburgh for sample collection and testing. In addition, we would like to thank Baxter Healthcare Corporation for providing the Isolex® reagents and the EMMES Corporation for advice on the statistical analysis of the data.

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Figure 1. CD34⁺ cell enumeration using an ISHAGE-based gating strategy

Viability was maintained throughout the different stages of the study. Representative plots from samples of a) fresh marrow, b) post-CD34⁺ cell selection product, and c) post-shipment product are shown. (Courtesy of D. McKeon, Immunophenotyping and Flow Cytometry Laboratory, University of Minnesota Medical Center.)

Figure 2. Summary of Flow Analysis by Processing Stage for All Facilities

Complete analysis for all centers for Phase I and Phase II is depicted based on data shown in Tables 1a, 1b and 2a. Samples were tested for B (CD19) and T (CD3) cell content as well as CD133/CD34 co-expression.

Table 1

Table 1a - Phase I Sample Results. Small volume sample (mean 58.1 mL) nucleated cell and CD34 ⁺ cell data are summarized.													
		Initial Results				Post-Isoplex® Selection Results					Post-Shipping Results		
Sample #	Beads	Starting Volume (mL)	Total Nucleated Cells	CD34 (%)	CD34 Count	Total Nucleated Cells	CD34 (%)	CD34 Count	CD34 Recovery (%)	Total Nucleated Cells	CD34 (%)	CD34 Count	* CD34 Recovery (%)
1	Full	57.7	1.03 × 10 ⁹	0.75	7.73 × 10 ⁶	1.08 × 10 ⁷	87.52	9.45 × 10 ⁶	122.3	1.50 × 10 ⁷	82.5	1.24 × 10 ⁷	205.9
2	Half	51	1.72 × 10 ⁹	0.69	1.19 × 10 ⁷	1.43 × 10 ⁷	84.67	1.21 × 10 ⁷	101.7	8.84 × 10 ⁶	81.43	7.20 × 10 ⁶	104.7
3	Half	62.4	1.37 × 10 ⁹	0.4	5.50 × 10 ⁶	9.00 × 10 ⁶	56.88	5.12 × 10 ⁶	93.1	4.12 × 10 ⁶	50.34	2.07 × 10 ⁶	76.4
4	Full	61.4	1.35 × 10 ⁹	0.39	5.25 × 10 ⁶	8.16 × 10 ⁶	63.84	5.21 × 10 ⁶	99.2	2.24 × 10 ⁶	51.35	1.15 × 10 ⁶	40.2
Mean		58.1	1.37 × 10 ⁹	0.58	7.60 × 10 ⁶	1.06 × 10 ⁷	73.23	7.97 × 10 ⁶	104.1	7.55 × 10 ⁶	66.41	5.71 × 10 ⁶	106.8

Table 1b - Phase I Sample Results. Large volume sample (mean 105.9 mL) nucleated cell and CD34 ⁺ cell data are summarized.													
		Initial Results				Post-Isotex® Selection Results				Post-Shipping Results			
Sample #	Beads	Starting Volume (mL)	Total Nucleated Cells	CD34 (%)	CD34 Count	Total Nucleated Cells	CD34 (%)	CD34 Count	CD34 Recovery (%)	Total Nucleated Cells	CD34 (%)	CD34 Count	*CD34 Recovery (%)
5	Full	97.4	3.43 × 10 ⁹	0.81	2.78 × 10 ⁷	4.16 × 10 ⁷	88.36	3.68 × 10 ⁷	132.4	2.27 × 10 ⁷	95.05	2.16 × 10 ⁷	77.4
6	Half	88.8	1.59 × 10 ⁹	0.5	7.97 × 10 ⁶	9.90 × 10 ⁶	93.43	9.25 × 10 ⁶	116.1	6.18 × 10 ⁶	92.87	5.74 × 10 ⁶	118.1
7	Full	116.3	2.98 × 10 ⁹	0.34	1.01 × 10 ⁷	1.52 × 10 ⁷	90.28	1.37 × 10 ⁷	135.6	5.61 × 10 ⁶	88.93	4.99 × 10 ⁶	94.5
8	Half	106.8	1.70 × 10 ⁹	1.3	2.21 × 10 ⁷	1.01 × 10 ⁷	96.11	9.71 × 10 ⁶	43.9	1.08 × 10 ⁷	91.8	9.91 × 10 ⁶	210.4
9	Full	120	5.05 × 10 ⁹	0.91	4.60 × 10 ⁷	4.37 × 10 ⁷	93.39	4.08 × 10 ⁷	88.7	2.69 × 10 ⁷	93.89	2.53 × 10 ⁷	81.4
Mean		105.9	2.95 × 10 ⁹	0.77	2.28 × 10 ⁷	2.41 × 10 ⁷	92.31	2.21 × 10 ⁷	103.3	1.44 × 10 ⁷	92.51	1.35 × 10 ⁷	116.4

Table 1c - Phase I Sample Results. Remaining QC testing (i.e., viability, CFU, and sterility) results for all samples are summarized.												
Initial Results				Post-Isoplex® Selection Results					Post-Shipment Results			
Sample #	Viability (%)	CFU/10 ⁶	Sterility	Viability (%)	CFU/10 ⁶	Sterility	Viability (%)	Sterility	Viability (%)	CFU/10 ⁶	Sterility	Sterility
1	94.96	975	No Growth	97.65	>2500	No Growth	93.94	No Growth	93.94	2400	No Growth	No Growth
2	99.01	1600	No Growth	99.14	>5000	No Growth	99.12	No Growth	99.12	1525	No Growth	No Growth
3	99.15	800	No Growth	99.30	>10000	No Growth	98.26	No Growth	98.26	3500	No Growth	No Growth
4	94.76	750	No Growth	98.16	>6667	No Growth	95.88	No Growth	95.88	6667	No Growth	No Growth
5	99.30	2100	No Growth	95.70	7700	No Growth	99.5	No Growth	99.5	6000	No Growth	No Growth
6	99.73	800	No Growth	99.07	>5000	No Growth	97.41	No Growth	97.41	>5000	No Growth	No Growth
7	99.39	1425	No Growth	99.17	>5000	No Growth	97.74	No Growth	97.74	TNP*	No Growth	No Growth

Table 1c - Phase I Sample Results. Remaining QC testing (i.e., viability, CFU, and sterility) results for all samples are summarized.										
	Initial Results			Post-Isoplex® Selection Results			Post-Shipment Results			
8	99.14	1200	No Growth	99.61	>5000	No Growth	97.94	>5000	No Growth	
9	97.85	1075	No Growth	98.93	3850	No Growth	99.12	3950	No Growth	
Mean	98.1	1192		98.5	>5635		97.7	>4255		

* CD34 % post-shipping recovery was determined after accounting for quality control testing and sample dilution prior to shipment (data not shown).

* CD34 % post-shipping recovery was determined after accounting for quality control testing and sample dilution prior to shipment (data not shown).

* TNP- Test not performed

Table 2

Table 2a - Phase II Sample Results. Nucleated cell and CD34 ⁺ cell data are summarized.												
Initial Results			Post-Isoplex Selection Results				Post-Shipping Results					
Sample #	Starting Volume (mL)	Total Nucleated Cells	CD34 (%)	CD34 Count	Total Nucleated Cells	CD34 (%)	CD34 Count	CD 34 Recovery (%)	Total Nucleated Cells	CD34 %	CD34 Count	* CD34 Recovery (%)
11	109.8	2.34 × 10 ⁹	1.49	3.49 × 10 ⁷	4.00 × 10 ⁷	75.15	3.01 × 10 ⁷	86.2	TNP	75.69	TNP	TNP
12	95.1	3.18 × 10 ⁹	2.98	9.47 × 10 ⁷	3.92 × 10 ⁷	95.28	3.73 × 10 ⁷	39.4	1.88 × 10 ⁷	95.61	1.80 × 10 ⁷	94.6
13	109.3	3.83 × 10 ⁹	1.60	6.12 × 10 ⁷	6.93 × 10 ⁷	76.51	5.30 × 10 ⁷	86.6	3.29 × 10 ⁷	67.01	2.21 × 10 ⁷	80.9
14	112.3	2.67 × 10 ⁹	0.85	2.27 × 10 ⁷	2.02 × 10 ⁷	84.79	1.71 × 10 ⁷	75.3	9.70 × 10 ⁶	86.40	8.38 × 10 ⁶	96.1
15	110	1.74 × 10 ⁹	0.50	8.69 × 10 ⁶	1.38 × 10 ⁷	57.04	7.86 × 10 ⁶	90.5	7.08 × 10 ⁶	47.95	3.40 × 10 ⁶	43.3
16	112	2.12 × 10 ⁹	0.35	7.48 × 10 ⁶	1.23 × 10 ⁷	56.60	6.94 × 10 ⁶	92.8	2.52 × 10 ⁶	66.70	1.68 × 10 ⁶	65.7
17	114	1.33 × 10 ⁹	0.66	8.78 × 10 ⁶	1.07 × 10 ⁷	63.30	6.76 × 10 ⁶	77.0	2.59 × 10 ⁶	58.10	1.51 × 10 ⁶	62.5
18	115	2.15 × 10 ⁹	0.52	1.12 × 10 ⁷	1.20 × 10 ⁷	62.30	7.45 × 10 ⁶	66.5	3.56 × 10 ⁶	70.40	2.51 × 10 ⁶	75.0
Mean	109.7	2.42 × 10 ⁹	1.12	3.12 × 10 ⁷	2.72 × 10 ⁷	71.37	2.08 × 10 ⁷	76.8	1.10 × 10 ⁷	70.98	8.23 × 10 ⁶	74.0
Table 2b Phase II- Pittsburgh reanalysis of samples 15–18												
Initial Results			Post-Isoplex Selection Results				Post-Shipping Results					
Sample #	Starting Volume (mL)	Total Nucleated Cells	CD34 (%)	CD34 Count	Total Nucleated Cells	CD34 (%)	CD34 Count	CD34 Recovery (%)	Total Nucleated Cells	CD34 %	CD34 Count	* CD34 Recovery (%)
11	109.8	2.34 × 10 ⁹	1.49	3.49 × 10 ⁷	4.00 × 10 ⁷	75.15	3.01 × 10 ⁷	86.2	TNP	75.69	TNP	TNP
12	95.1	3.18 × 10 ⁹	2.98	9.47 × 10 ⁷	3.92 × 10 ⁷	95.28	3.73 × 10 ⁷	39.4	1.88 × 10 ⁷	95.61	1.80 × 10 ⁷	94.6
13	109.3	3.83 × 10 ⁹	1.60	6.12 × 10 ⁷	6.93 × 10 ⁷	76.51	5.30 × 10 ⁷	86.6	3.29 × 10 ⁷	67.01	2.21 × 10 ⁷	80.9
14	112.3	2.67 × 10 ⁹	0.85	2.27 × 10 ⁷	2.02 × 10 ⁷	84.79	1.71 × 10 ⁷	75.3	9.70 × 10 ⁶	86.40	8.38 × 10 ⁶	96.1
15	110	1.83 × 10 ⁹	0.50	9.16 × 10 ⁶	1.67 × 10 ⁷	56.25	9.38 × 10 ⁶	102.42	1.57 × 10 ⁷	47.79	7.50 × 10 ⁶	79.9
16	112	2.31 × 10 ⁹	0.36	8.30 × 10 ⁶	9.20 × 10 ⁶	55.15	5.07 × 10 ⁶	61.08	6.48 × 10 ⁶	67.61	4.38 × 10 ⁶	86.4
17	114	1.17 × 10 ⁹	0.66	7.70 × 10 ⁶	1.30 × 10 ⁷	63.32	8.20 × 10 ⁶	106.51	1.73 × 10 ⁷	59.29	1.03 × 10 ⁷	125.4
18	115	2.21 × 10 ⁹	0.53	1.17 × 10 ⁷	1.22 × 10 ⁷	62.87	7.65 × 10 ⁶	65.28	2.55 × 10 ⁷	68.07	1.74 × 10 ⁷	227.0
Mean	109.7	2.44 × 10 ⁹	1.12	3.13 × 10 ⁷	1.63 × 10 ⁷	71.2	2.10 × 10 ⁷	77.8	1.81 × 10 ⁷	70.9	1.26 × 10 ⁷	112.9

* CD34 % post-shipping recovery was determined after accounting for quality control testing and sample dilution prior to shipment (data not shown).

* CD34 % post-shipping recovery was determined after accounting for quality control testing and sample dilution prior to shipment (data not shown).