# BioSciences Research Associates, Inc.

767c Concord Ave. Cambridge, MA 02138  Phone: 617 441-2050 Fax: 617 441-2052


## Preliminary Report

Prepared for:
Emery Smith
Emcyte Corporation
August 20, 2006

# Analysis of GenesisCS: Human Bone Marrow Concentration


Sherwin V. Kevy, M.D.
Associate Professor Pediatrics
Harvard Medical School
Consultant in Hematology and Director Emeritus
Transfusion Services
Children's Hospital Boston, MA

May S. Jacobson, Ph.D
Research Associate in Medicine (Hematology)
Children's Hospital Boston, MA

Robert J. Mandle, Ph.D.
Investigator
The CBR Institute for Biomedical Research
Boston, MA
President
BioSciences Research Associates, Inc.
Cambridge, MA

Prepared by: Robert J. Mandle, Ph.D.

**Research Study Plan**

| Title: **Evaluation of GenesisCS with bone marrow aspirate** | |
|---|---|
| Principle Investigator(s): Dr. Sherwin Kevy[1,2], Dr. May Jacobson[1,2] and Dr. Robert Mandle[2,3].<br>1. Children's Hospital, Harvard Medical School<br>2. The CBR Institute for Biomedical Research<br>3. Biosciences Research Associates, Boston, MA | |
| Date Initiated:<br>16 August 2006 | Projected Completion Date:<br>22 Aug 2006 |
| Revision if applicable (A-Z): | Revision Date: |

Type of Study: Design Verification Regulatory Submission Market Support Manufacturing Verification X investigative

Objective of Study: Preliminary evaluation of GenesisCS for concentration of human bone marrow aspirate.

**Introduction:**

Preclinical and clinical studies have suggested the benefit of using concentrated autologous bone marrow aspirate in bone repair, myocardial infarct and peripheral vascular disease. Bone marrow aspirate is often not sufficient for clinical efficacy in the absence of concentration[1,2]. This report represents results from an evaluation of GenesisCS device for the concentration of human bone marrow-derived stem cells. Sixty mL of human bone marrow aspirate were concentrated to approximately 5 ml with the GenesisCS. Samples of the bone marrow aspirate (BMA) and resulting bone marrow concentrate (BMC) were analyzed for Total Nucleated Cells (TNC), Platelets (Plt), and CD34 positive Hematopoietic Stem Cells (HSC). Yield calculation were done for TNC, Plt and HCS.

**Method:**

Donor bone marrow samples, approximately 120mL, collected from two sites of the iliac crest, were obtained from Poetics (Cambrex). Bone marrow samples were collected in 30-50 units/mL of heparin. Processing and all testing were initiated within 24 hr of collection.

After obtaining a 1mL start sample from a well mixed transfer pack of BMA, two 60 mL syringes were filled with approximately 60 ml of marrow aspirate and the volumes recorded. GenesisCS disposables were filled from these syringes through the luer-lock fitting at a fill rate of approximately 1 mL/sec. Disposables were centrifuged at 2400 rpm (1020 x g) for 12 min. Two independent centrifuge runs were performed for each donor BMA from two separate donors collected on separate days for a total of four runs. Following centrifugation, the plasma layer was removed, by lowering the collection head to within 2-5mm above the buffy coat layer which contained the concentrated nucleated

cells and platelets. Next, the remaining plasma and an additional 4 mL of the buffy coat was removed (4 ml following the first flash of RBC observed in the suction tubing above the collection device).

Analysis of BMA and BMC consisted of:

- Complete blood counts utilizing a Medtronic 620 -16 parameter hematology analyzer with extended platelet range.
- Cytometric analysis of CD34 positive hematopoietic stem/progenitor cells
- Manual differential counts on BMA and BMC samples.
- Yield of nucleated cells, platelets and CD34 positive HSCs were calculated for bone marrow concentrates

**Results:**

**Characterization of GenesisCS BMC:**

The TNC values from the hematology analyzer for pre-sample (BMA) and for product (BMC) and the calculated concentration over baseline vaues are shown in Table I.

Table I: Total nucleated cells (2 donors with duplicate runs)

| | Volume | Total Nuclear Cells x $10^3/\mu L$ | Concentration Above Baseline |
|---|---|---|---|
| Bone Marrow Aspirate | 60 | 16-23 | 1.0x |
| Bone Marrow Concentrate | 4 | 170-271 | 11.5x |

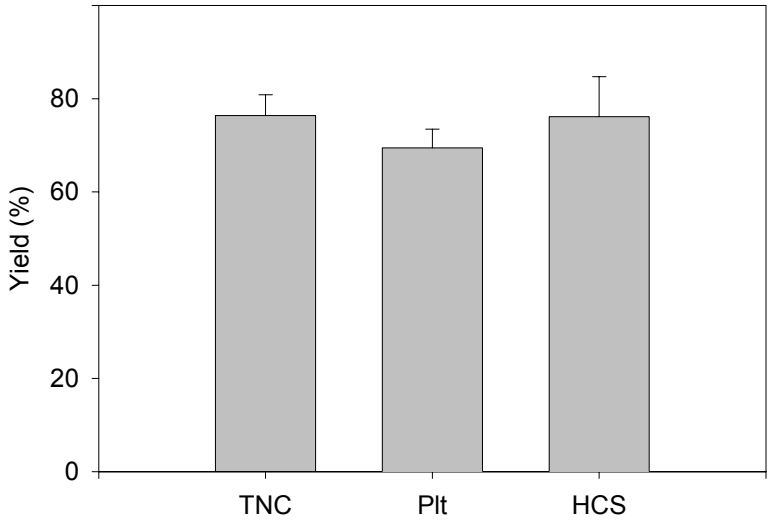
Table II lists the calculated total number of cells (volume x concentration) in BMA and BMC . TNC and Plt counts represent the values from the hematology analyzer times the volumes of BMA or BMC. HSC numbers are calculated from the percent of $CD34^+$ cells gated with CD45+ events times the number of WBC (TNC minus nucleated red blood cells).

Table II: Total cell numbers ± SD (volume x cell concentration) 2 donors with replicates

| Bone Marrow Aspirate | | | Bone Marrow Concentrate | | |
|---|---|---|---|---|---|
| TNC x $10^6$ | HSC x $10^6$ | Plt x $10^6$ | TNC x $10^6$ | HSC x $10^6$ | Plt x $10^6$ |
| 1161 ± 239 | 9.0 ± 1.3 | 10,830 ± 1836 | 894 ± 232 | 6.9 ± 1.4 | 7,623 ± 1432 |
| Concentration factor (times baseline) In 4ml buffy coat BMC | | | 11.6 | 11.5 | 10.6 |

The recovery of TNC, Plt and $CD34^+$ HCS were calculated and are shown in Figure 1.

Figure 1. Recovery of TNC, Plt and $CD34^+$ HCS




The percent of TNC, Plt, and $CD34^+$ HSC were calculated by dividing the total number of cells recovered in the BMC by the total number present in 60ml of BMA and are represented as mean plus standard deviation for 2 donors with duplicate runs.

**Summary and conclusions:**

The product (BMC) yields were 76% for TNCs and $CD34^+$ HSC. These yields are consistent with other point of care bone marrow concentrating devices. Platelet yields in the BMC averaged 70% and the product Hematocrit averaged 31.6% with a range of 31-40% (data not shown). Hematocrit can be adjusted by including more or less of the plasma layer during the collection of BMC. Variation within donor samples appears to less than between donors. Between donor variation will need to be determined in a larger study. However, the data from this preliminary evaluation with two donors run in duplicate, is very encouraging.

**References:**

1. Saigaw, T, et al, "Clinical Application of Bone Marrow Implantation in Patients with Arteriosclerosis Obliterans, and the Association between Efficacy and the Number of Implanted Bone Marrow Cells, *Circulation Journal*, 68(12):1189-1193, 2004
2. Hernigou, P.H., "Percutaneous Autologous Bone Marrow Grafting for Nonunions. Influence of the Number and Concentration of Progenitor Cells", *Journal of Bone & Joint Surgery*, 97-A:1430-1437, 2005