

Supplemental Methods Section

Measures of Perfusion.

The ABI was obtained by measuring the posterior tibial and dorsalis pedal arteries and the highest brachial artery pressure, and the first digital artery pressure was used for FTP and TBI. The FTP, TBI, and ABI were recorded by the same technician at each follow-up visit in a temperature controlled room after 15 minutes of rest. TcPO₂ measurements were recorded by means of a sensor placed on the anterior chest wall as a reference and on the dorsum of the foot for analysis.

Bone Marrow Aspiration and ABMNC Isolation. In the prone position and under general anesthesia bone marrow (BM) (360-370 ml.) was aspirated from the right and left posterior iliac crests. With the Ficoll density centrifugation (FC) technique the patient was recovered from general anesthesia as the ABMNCs were isolated in the stem cell laboratory and later returned to the operating room for cell injection. Using the MarrowStim™ (MS) centrifuge system, BM was loaded into the centrifuge tube and the buffy coat was extracted (**Fig.1 A-C**). The average number of ABMNCs obtained for the FC isolation was $1.3 \pm 0.7 \times 10^9$ and for the MS isolation was $2.0 \pm 1.6 \times 10^9$, respectively. Cell viability was greater than 97% for both FC and MS. None of the cell preparations were contaminated as determined by gram stain, bacterial cultures, and endotoxin assays that were obtained prior to cell administration. The ABMNC suspension for FS and MS was concentrated to a volume of 30 ml. and 0.75 ml. aliquots were delivered via IM injections at 2 cm intervals, 1.5 cm deep into the gastrocnemius muscle, along the medial and lateral aspect of the index limb. In those patients with distal occlusive disease extending below the malleoli (based on imaging studies prior to enrollment) injections were extended onto the

dorsal and medial aspects in the distribution of the dorsalis pedal and posterior tibial arteries and terminal arcades. Injections of ABMNCs (number of injections, mean 37 ± 6) were delivered starting proximally at the level of the anterior tibial tuberosity and continued onto the dorsum of the foot (**Fig.1D**).

Flow Cytometric Characterization of Endothelial Progenitor Cell Subpopulations.

Fluorescent activated cell sorting (FACS) analysis was used to quantify subpopulations of EPCs within the MNC fraction prior to injection in eight patients enrolled in the study. Using recognized surface markers for EPCs¹⁷⁻¹⁹, MNCs were stained with different primary or isotype control antibodies at 4°C for 30 minutes in 100 μ L phosphate buffered saline (PBS) containing 2% fetal bovine serum, washed twice with PBS, fixed with 1% paraformaldehyde, and analyzed using a Calibur flow cytometer and Cell QuestPro software (Becton Dickinson Immunocytometry Systems, San Diego, CA). The following primary anti-human murine monoclonal antibodies were used (all BD Pharmingen, San Diego, CA, unless indicated); CD 133-phycoerythrin (PE), CD45- fluorescein isothiocyanate (FITC), CD 34-FITC, IgG1-FITC (isotype control), and IgG1-PE (isotype control), KDR -PE (R & D Systems, Minneapolis, MN).