Final Report
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Noninvasive Imaging of Administered Progenitor Cells
PI: Steven R. Bergmann M.D., Ph.D.
Beth Israel Medical Center
New York, NY

The objective of this research grant was to develop an approach for labeling progenitor cells, specifically those that we had identified as being able to replace ischemic heart cells, so that the distribution could be followed non-invasively. In addition, the research was aimed at determining whether administration of progenitor cells resulted in improved myocardial perfusion and function. The efficiency and toxicity of radiolabeling of progenitor cells was to be evaluated.

For the proposed clinical protocol, subjects with end-stage ischemic coronary artery disease were to undergo a screening cardiac positron emission tomography (PET) scan using N-13 ammonia to delineate myocardial perfusion and function. If they qualified based on their PET scan, they would undergo an in-hospital protocol whereby CD34+ cells were stimulated by the administration of granulocytes-colony stimulating factor (G-CSF). CD34+ cells would then be isolated by apharesis, and labeled with indium-111 oxine. Cells were to be re-infused and subjects were to undergo single photon emission computed tomography (SPECT) scanning to evaluate uptake and distribution of labeled progenitor cells. Three months after administration of progenitor cells, a cardiac PET scan was to be repeated to evaluate changes in myocardial perfusion and/or function.

Indium oxine is a radiopharmaceutical for labeling of autologous lymphocytes. Indium-111 (In-111) decays by electron capture with a t½ of 67.2 hours (2.8 days). Indium forms a saturated complex that is neutral, lipid soluble, and permeates the cell membrane. Within the cell, the indium-oxyquinolone complex labels via indium intracellular chelation. Following leukocyte labeling, ~77% of the In-111 is incorporated in the cell pellet. The presence of red cells and /or plasma reduces the labeling efficacy. Therefore, the product needed to be washed to eliminate plasma proteins. This repeated washing can damage cells. The CD34 selected product was a 90-99% pure population of leukocytes. Viability was assessed using Trypan blue histological analysis.

We successfully isolated and labeled ~25-30 x 10^{7} CD34+ lymphocytes in cytokine mobilized progenitor cell apharesis harvests. Cells were also subjected to a stat gram stain to look for bacterial contamination, stat endotoxin LAL to look for endotoxin contamination, flow cytometry for evaluation of the purity of the cells and 14-day sterility culture.

Colony forming assays confirm the capacity of these cells to proliferate and function ex-vivo with CFU-GM values of 26 colonies/ 1 x 10^{4} cells plated and 97% viability in cytokine augmented methylcellulose at 10-14 days in CO_2 incubation. We developed a closed-processing system for the product labeling prior to infusion to maintain

autologous cell integrity and sterility. Release criteria for the labeled product were documented for viability, cell count and differential, and measured radiolabel.

We were successful in labeling the cells with up to 500 uCi/10^8 cells, with viability of >98%. However, due to delays in getting the protocol approved by the FDA, the cells were not infused in humans in this location (although we did successfully use CD34+ cells in humans in a study in Australia).

The approach developed should permit labeling of progenitor cells that can be administered to human subjects for tracking. The labeling approach should be useful for all progenitor cell types, although this would need to be verified since different cell lines may have differential radiosensitivity.