

The objective of this pilot study is to determine and compare the efficacy of posterior spine fusion with different sources of mesenchymal stem cells that are transduced with BMP2, BMP6, and BMP9. Robust bone regeneration has important implications for the clinical management of many musculoskeletal disorders, including bone grafting, fracture healing and spinal fusion. Two Specific Aims were proposed in the original application. The first aim was to determine the transduction efficiency of adenoviral vectors expressing BMP2, BMP6 and BMP9 in rabbit mesenchymal stem cells. The second aim was to determine the efficacy of posterior spine fusion mediated by the AdBMP-transduced mesenchymal stem cells derived from bone marrow and peripheral blood cells.

Since receiving the NASS funding, we have completed the first Aim. Specifically, recombinant adenoviruses (AdBMPs) expressing BMP2, BMP6 and BMP9 were used to infect rabbit mesenchymal stem cells derived from either bone marrow cells (BMCs) or peripheral buffy-coat cells (PBCs). By monitoring the expression of green fluorescence protein (GFP), we found that both marrow cells and buffy coat cells were effectively transduced by the AdBMP2, 6, and 9 vectors, and the gene expression lasted at least 10 days under cell culture conditions.

We next carried out a pilot animal study, in which autologous BMCs and PBCs were first transduced *ex vivo* with AdBMP2, 6, and 9, and the AdGFP control, and the transduced cells were soaked in Type I collagen sponges and placed over the decorticated costal processes of rabbit lumbar L4-5 vertebrae to complete the arthrodesis. The level and duration of transgene expression were determined by detecting the GFP expression postmortem at 2, 4, and 8 weeks after implantation. The fusion sites were examined radiographically and histologically.

Since the submission of our Status Report in August 2003, we have conducted more animal studies to determine the reproducibility and efficacy of BMP2, 6 and 9-mediated spinal fusion, with emphasis on using BMCs as cell carriers. Through x-ray radiographic, CT scan and histological examination, we demonstrated that all three BMPs can effectively induce spinal fusion using transduced bone marrow cells, at as early as four weeks after implantation. These findings confirm and expand our pilot studies described in the previous Status Report.

### **Findings or Conclusions to Date**

Our results demonstrate that: 1) AdBMPs effectively transduced both BMCs and PBCs; 2) all three BMPs were able to induce solid spinal fusion at as early as four weeks after implantation; and 3) BMCs were better recipient (than PBCs) of gene transfer and promoted more effective spinal fusion.