

Efficient Transduction of Marrow Progenitors and Long-term Gene Expression in their Progeny by *in Vivo* Gene Delivery

A.A. Chekmasova¹, J.P. Louboutin¹, M. Singh¹, D.S. Strayer¹

¹Thomas Jefferson University, Philadelphia, PA

Objective: To assess the effectiveness and longevity of hematopoietic stem cell gene delivery by direct intramarrow inoculation.

Methods: Recombinant SV40-derived vectors (rSV40) were used to deliver transgenes to rabbit hematopoietic progenitor cells *in vivo* as a model for stem cell-directed gene therapy. A bifunctional vector, SV (RNAiR5-RevM10.AU1) carries RevM10 with a carboxyl terminus AU1 epitope tag, and RNAiR5, an interfering RNA against CCR5. Transgene expression was tested in bone marrow, blood and different organs by flow cytometry (FACS) or fluorescence microscopy, both as RevM10 production using antibody against the AU1 epitope and as down-regulation of CCR5 by the anti-CCR5 RNAi, using antibody to detect cell membrane CCR5.

Results: About 30-35% of whole femoral bone marrow (BM) expressed AU1 two weeks after intrafemoral injection with SV (RNAiR5-RevM10.AU1), including 75.5% of cells in the granulocyte series and 61.5% of stem cell antigen (SCA)-positive cells. From 30 to 35% of peripheral blood mononuclear cells (PBMC) expressed the RevM10-AU1 transgene throughout this study, until it was terminated, 56 weeks post-injection. T-cells (CD3, CD4, CD8), B-cells, monocytes (bearing CD14) and granulocytes all expressed the delivered RevM10-AU1 protein. In addition, levels of PBMC cell membrane CCR5 were decreased by 30-50% throughout this time period. When the study was terminated 56 weeks post-injection, we tested expression of AU1 in BM and various organs of injected animals. 30-35% of femoral BM cells expressed AU1 at 56w. AU1-positive cells were detected in other marrow sources that had not been injected: humerus (11%); tibia (31%); and iliac crest (45%), including 32% of SCA+ cells in the humerus, 61.5% in the tibia, 78.4% in the iliac crest and 73.6% in the femur. Expression of RevM10-AU1 in the different organs varied and was greatest in the lungs and spleen.

Conclusion: rSV40s transduce nondividing HSC very efficiently, making transduction by direct intramarrow inoculation practicable. Thus, direct intramarrow administration of rSV40s *in vivo* transduces HSC and provides stable long-term transgene expression in high percentages of differentiated HSC progeny, in the blood and tissues.

Translational Applicability: Stable gene transfer to HSC *ex vivo* transduction with transplantation has been problematic. Direct intramarrow inoculation with rSV40 vectors provides long-term, stable transgene expression in HSC and their progeny without toxicity, *ex vivo* manipulation or transplantation. This approach may be useful in treating an array of inherited and acquired diseases of HSC and their derivatives.