

P039 A Combined Gene Therapy Approach to Axon Regeneration using Lentiviral Vector delivery of siRNA and Neurotrophin 3
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Chondroitin sulfate proteoglycan (CS-PG) expression is increased in response to CNS injury and forms the major component of the glial scar. We are investigating a novel approach to reduce the glial scar and promote axon regeneration by viral vector delivery of small inhibitory RNAs (siRNA) specifically targeting the proteoglycan NG2 and co-expression of NT3.

Preliminary data using two validated siRNA sequences targeted to NG2 demonstrated a 70% reduction in mRNA expression using RT-PCR, 48 hrs post transfection of the Neu 7 astrocyte cell line. NG2 protein expression, assessed by immunostaining was also reduced. The complementary DNA oligonucleotide sequences of the NG2 siRNA were cloned into both a plasmid and lentiviral vector based expression system. Transduction of Neu7 cells with a HIV-1-GFP lentiviral vector demonstrated up to 80% transduction efficiency. Transduction of Hela cells with an NT3 expressing lentiviral vector demonstrated up 500 pg/ml of NT3 expressed in the supernatant. Mouse p19 cell differentiated into neurons with retinoic acid and then transduced with the NT3 lentiviral vector, demonstrated levels of 60 to 80 pg/ml. The NT3 lentiviral vector could be used to transduce transplantable stem cells to aid axon regeneration in combination with siRNA expression.