**Introduction:** Mucopolysaccharidosis type VI (MPS VI) is an inherited metabolic disorder that arises from a deficiency or total absence of the lysosomal enzyme N-acetylgalactosamine-4-sulphatase (4S) required for the intracellular degradation of chondroitin sulphate (CS) and dermatan sulphate (DS) glycosaminoglycan chains. Undegraded CS and DS thus accumulate in a wide range of cells and tissues leading to multiple organ failure. The cardinal feature of MPS VI is a progressive skeletal dysplasia that manifests as reduced bone mass, short stature, stiff joints and degenerative joint disease. Other symptoms include an enlarged liver and spleen, cardiomyopathy and valvular dysfunction, and corneal clouding. Gene therapy offers the potential to provide sustained, high levels of enzyme in the MPS VI joint via gene transfer to affected cells. This study was undertaken to evaluate the efficacy of a lentiviral vector delivering recombinant human 4S to infect various joint tissues in vitro and to infect different tissues in the joint (cartilage, synovium and ligament) in vivo, after direct injection into the joint space.

**Materials and Methods:** A lentiviral vector was constructed consisting of an HIV-1 backbone and containing the 4S sequence under the transcriptional control of the murine phosphoglycerate kinase gene (pgk) promoter. pHIV-1pgkLacZnlsco and pHIV-1mpsvLacZnlsco are analogous constructs containing a codon optimized LacZ gene under the transcriptional control of the pgk or the myeloproliferative sarcoma virus long terminal repeat promoter (mpsv) respectively. Virus was produced by calcium phosphate transfection of 293T cells with one of the above constructs and accessory plasmids. Medium containing virus was collected and concentrated by centrifugation. The pellet was resuspended in phosphate buffered saline and the concentration determined by ELISA for HIV-1 p24 protein. MPS VI skin fibroblasts or chondrocytes were incubated with (i) different doses of (0-273 ng of p24 protein) of pHIV-1pgk4S for 6 days or with (ii) pHIV-1pgk4S (273 ng of p24 protein) for up to 41 days. Removal of accumulated CS and DS was determined by incubating cells with 35SO4, followed by transfection with pHIV-1pgk4S. Viral copy number was determined by PCR of genomic DNA to detect the gag sequence present in the vector and 4S activity was measured using the fluorogenic substrate, 4-methylumbelliferyl sulphate. The efficacy of 2 different promoters to drive the expression of β-galactosidase was determined by infecting normal chondrocytes or synovial membrane fibroblasts with pHIV-1pgkLacZnlsco or pHIV-1mpsvLacZnlsco. After 7 days the cells were harvested with trypsin/EDTA and assayed for β-galactosidase using the Roche chemiluminescence kit. Normal rat knees were injected with pHIV-1mpsvLacZnlsco (corresponding to 70ng – 7 µg p24 protein) and left for up to 12 weeks. After sacrifice, the knee joints were removed and fixed in toto in 2% formaldehyde, 0.2% glutaraldehyde for 15 min, followed by staining in X-gal solution for 5 hrs. Separate tissues were then removed and fixed in 10% buffered formalin prior to sectioning and counterstained with H&E.

**Results:** With increasing dose of pHIV-1pgk4S, virus copy number and 4S activity in transduced MPS VI skin fibroblasts increased. In the absence of virus, untransduced MPS VI cells contained 4S activity equivalent to 7 % of normal cell activity and this increased to 12 times the normal value when MPS VI cells were transduced with the highest dose of pHIV-1pgk4S tested (273 ng p24 protein). Concomitant with the increase in cellular 4S activity observed after viral transduction a decrease in 35SO4-labeled CS and DS chains was observed in MPS VI cells. Expression of 4S from the pHIV-1pgk4S vector was observed for up to 41 days in MPS VI skin fibroblasts, with maximal expression observed between days 12 and 26. Virus copy number decreased steadily with time in culture. The comparison of 2 different promoters, the pgk and mpsv promoters, demonstrated a higher level of marker gene expression driven by the mpsv promoter in both chondrocytes and synoviocytes. Based on the increased marker gene expression from pHIV-1mpsvLacZnlsco in vitro, this construct was injected into the rat knee to assess the ability of the lentiviral vector to infect various joint tissues in vivo. One week after injection with pHIV-1mpsvLacZnlsco corresponding to 7 µg p24 protein, the synovial membrane stained strongly for β-galactosidase. However, no staining of cartilage chondrocytes or ligament fibroblasts was observed. Dilution of the virus resulted in a more restricted staining pattern. Transduced synovial cells were still clearly observed 4 weeks after injection, however, the number and distribution of transduced cells was greatly reduced 8 weeks after injection and no transduced cells were observed 12 weeks after injection.

**Discussion:** The lentiviral vector readily transduced skin fibroblasts, chondrocytes and synovial membrane fibroblasts in cell culture. In MPS VI cells the level of 4S expression corresponded to the dose of lentiviral vector administered and efficiently removed the accumulated CS and DS glycosaminoglycan chains. When injected into the joint the lentivirus readily transduced cells in the synovial membrane but not cartilage chondrocytes or ligament fibroblasts. Thus we anticipate that any use of this viral system as a treatment for joint disease in MPS VI will rely on a localised ERT effect. That is, overproduction of 4S by transduced synovial cells resulting in increased enzyme levels in the synovial fluid will promote diffusion into cartilage and ligament and uptake by their resident cells. The expression of β-galactosidase from the mpsv promoter persisted for at least 8 weeks in vivo, albeit at a reduced level compared to earlier times. We would expect that the functional effect (equates to removal of accumulated CS/DS) of expression of 4S from an analogous vector would be considerably longer than 8 weeks. That is, after expression has ceased the synthesised enzyme will continue to have an effect that correlates to its intrinsic tissue half-life. From previous studies, re-accumulation of CS and DS after a single injection of recombinant 4S took approximately 4 weeks. Thus the effect of 4S delivery using the lentiviral system will persist longer than direct injection of recombinant 4S. This will provide a significant advantage to MPS VI patients by reducing the number and frequency of joint injections.