Mucopolysaccharidoses (MPS) are a group of 11 inherited metabolic disorders arising from a deficiency in a lysosomal protein required for the degradation of glycosaminoglycans (GAG) carbohydrates. MPS type VII (MPS VII) is an autosomal recessive lysosomal storage disorder caused by β-glucuronidase deficiency resulting in the accumulation of chondroitin sulphate, heparan sulphate and dermatan sulphate gags in a variety of cell types. MPS VII is one of the 7 MPSs types that display predominantly skeletal pathology. Short stature, alterations in bone mass and angulation deformities are all typical features of bone disease in MPS and are collectively termed dysostosis multiplex. The progressive worsening of skeletal pathology with age is a major cause of medical consultations and requires ongoing orthopaedic intervention to maintain some degree of independent movement.

The naturally occurring MPS VII mouse (GUS"/"mice strain) has no residual β-glucuronidase activity and represents the severe end of the clinical spectrum. It displays a phenotype typical of the human disorder, most notably a severe, progressive skeletal dysplasia.

In this study we evaluated a lentiviral-mediated gene therapy protocol for its efficacy in preventing the development and progression of bone pathology in MPS VII.

Methods:
MPS VII (GUS"/"mice) were bred from heterozygous parents and genotype determined by PCR. A lentiviral vector (pHIV-1EF1β-gluc) consisting of an HIV-1 lentiviral backbone containing the murine β-glucuronidase gene under the transcriptional control of the elongation 1 alpha (EF1α) promoter was used to produce virus. An analogous vector, pHIV-1EF1αLacZ, containing a reporter gene was also produced. A dose corresponding to 5µg p24 virus protein (pHIV-1EF1β-gluc or pHIV-1EF1αLacZ) was injected into the superficial temporal vein 2 days after birth. After 7 days mice injected with the reporter gene were killed to determine virus distribution. At 6 months of age mice treated with pHIV-1EF1β-gluc were killed, tissues harvested and enzyme activity assayed using a fluorogenic substrate. Quantitative bone parameters were measured on the L5 vertebra using CTAn analysis software. Results:

Vertebral bone mineral volume (BV/TV, Fig 2) in untreated MPS VII mice was significantly higher than normal (49.8±3.2% vs 37.7±1.9% respectively) and decreased in the gene therapy treated mice to 43.1±2.3%. An increase in trabecular number (TbN, Fig 3) was also observed in MPS VII (5.3±0.3 per mm vs 4.0±0.2 per mm in normal) and the number decreased in gene therapy treated mice to 4.5±0.2 per mm. Trabecular thickness (TbTh, Fig 4) was similar to normal (94±6µm in MPS VII vs 93±4µm in normal) and was not altered in the treated mice. Bone length decreased in MPS VII (Fig 5) from 2.7±0.1 mm to 3.4±0.2 mm in normal and did not change upon treatment. Gene therapy resulted in an increase in β-glucuronidase activity in all tissues examined and ranged from a 5x increase in the kidney to a 200x increase in liver.

Conclusion:
Bone pathology in MPS VII manifests as an increase in bone mass and a decrease in bone length. The former is due to the persistence of trabeculae that may result from impaired osteoclast function, which are unable to remove trabeculae during remodeling. The decrease in length suggests that the proliferation and hypertrophy of growth plate cells are thus accessible to enzyme. In contrast, cartilage cells within the growth plate are not accessible to enzyme as evidenced by the lack of improvement in vertebral height and the lack of transduction of cartilage cells (Fig 1). These preliminary results suggest that a systemic gene therapy approach may prove effective in reducing some of the bone changes exhibited by MPS VII patients but that different delivery strategies may be necessary to alter cartilage pathology.

Ongoing analysis is focussed on evaluating different doses and the age at which gene therapy is administered to maximise the bone cell response. Localised intra-articular administration of lentivirus is also being considered to improve transduction and or enzyme accessibility to cartilage cells.