Long-term Outcomes of Brain-targeted Stem Cell Gene Therapy for Mucopolysaccharidosis Type II in a Pre-Clinical MPSII Mouse Model.

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Introduction

Mucopolysaccharidosis type II (MPSII or Hunter Disease) is an X-linked recessive lysosomal storage disease and is caused by mutations in the IDS gene that codes for Iduronate-2-Sulfatase (IDS). IDS is an enzyme involved in the degradation of glycosaminoglycans (GAGs) such as heparan sulfate and dermatan sulfate, which build up throughout the body in the absence of IDS. Patients suffer from a range of symptoms including severe neurodegeneration, cardio-respiratory complications and skeletal abnormalities. Enzyme replacement therapy (ERT) and haematopoietic stem cell transplant (HSCT) are routinely used in the clinic to treat MPSII, however neither is efficacious at treating the neurological symptoms. ERT typically fails due to the inability of the enzyme to cross the blood-brain-barrier (BBB), whereas in HSCT, transplant derived cells cross the BBB and secrete enzyme. Unfortunately, the levels of native enzyme delivered by a normal HSCT are insufficient for disease correction in the brain.

Haematopoietic Stem Cell Gene Therapy (HSCGT), delivering the missing transgene to autologous HSCs, has the potential to overcome the deficiencies in enzyme expression (1).

We previously demonstrated that HSCGT with lentiviral IDS under a myeloid promoter fused to a BBB-crossing peptide (LV.IDS.ApoEII) can correct the disease phenotype in Ids-/- mice 6 months post-transplant, where native IDS (LV.IDS), only mediated a partial brain correction (2).

Here we demonstrate that neuropathological correction is maintained at 10-12 months post-HSCGT. This improvement in correction is seen despite the lower detectable vector copy number (VCN) and IDS activity in LV.IDS.ApoEII-treated animals compared to LV.IDS.
A bone marrow transplant was performed in MPSII mice with LV.IDS.ApoEII, LV.IDS and WT cells and compared to untreated MPSII mice and WT C57BL6 mice.

Figure 1. Biochemical Outcomes in MPSII Mice 12 Months Post-transplant.
- Supra-physiological levels of IDS and IDS.ApoEII enzyme in peripheral organs (A, B and D).
- Higher enzyme (via IDS activity assay) in the brain for IDS vs IDS.ApoEII (C).
- Higher vector copy number (VCN, determined via qPCR) for IDS vs IDS.ApoEII in bone marrow (E), spleen (F), brain (G) and the blood (H).
- Both IDS and IDS.ApoEII significantly reduce HS levels in MPSII mice (I).
- Both IDS and IDS.ApoEII normalised HS sulfation pattern (J).
Long-term (12 months post-transplant) Outcomes in MPSII Mice

- **Figure 2. Histochemical Staining of MPSII Mice Brain Sections 12 Months Post-transplant.**
  - LAMP2 staining indicates that lysosomal swelling is reduced by both IDS and IDS.ApoEII, however greater reduction is seen with IDS.ApoEII.
  - GFAP staining indicates that astrocytosis is significantly reduced, in both the cortex and amygdala, by IDS and IDS.ApoEII. Greater correction of the amygdala was seen with IDS.ApoEII.
  - ILB4 staining indicates a significant reduction in microgliosis with both IDS and IDS.ApoEII, however IDS.ApoEII facilitated a significantly greater reduction.
Figure 3. H&E Staining of Retinal Sections from MPSII mice.
- H&E staining of retinal sections reveals a severe degeneration of the outer nuclear layer (ONL) in MPSII mice compared to wild-type controls.
- Treatment with IDS or IDS.ApoEII HSCGT preserves retinal structure at 12-months post-transplant.

Figure 4. H&E Staining of Liver and Kidney from MPSII mice.
- H&E staining of liver sections revealed accumulations of vacuolated macrophages within the interstitium, spreading to involve hepatocytes.
- In the kidney, vacuolation of tubular epithelium and glomeruli is seen in MPSII mice.
- These findings were attenuated with IDS.ApoEII HSCGT.
Figure 5. Transductions of human CD34+ Stem Cells.

- Transductions of human CD34+ stem cells (from mobilised peripheral blood mononuclear cells) revealed no significant differences between transduced and untransduced cells for total colony numbers and colony forming unit numbers following 14 days in methocellulose culture (A and B).
- IDS activity was equivalent between IDS and IDS.ApoEII when normalised to detectable VCN (C and D).

Conclusions

- Correction of peripheral disease is maintained at 12 months post-transplant.
- Sustained and superior correction with IDS.ApoEII over native IDS at 12 months post-transplant.
- IDS.ApoEII required less brain enzyme than IDS (3.7% vs 13%) to show neuropathological correction in MPSII mice 12 months post-transplant.
- HSCGT with IDS or IDS.ApoEII partially preserves retinal structure at 12 moths post-transplant.
- IDS.ApoEII corrects liver and kidney pathology.
- LV.IDS.ApoEII transductions are well tolerated in human CD34+ stem cells.

References