ZFN-Mediated *In Vivo* Genome Editing Results in Phenotypic Correction in MPS I and MPS II Mouse Models

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Mucopolysaccharidosis (MPS) types I & II result from lysosomal accumulation of dermatan & heparan sulfate

Glycosaminoglycans (GAGs)

*Iduronate 2-sulfatase (IDS/MPS II)*

*α-L-iduronidase (IDUA/MPS I)*

- MPS I is autosomal
- MPS II is X-linked
- Incidence is ~0.3-1 per 100,000 births
- Life expectancy in severe forms less than 10 years

Modified after Neufeld and Muenzer 2001 & Kowalewski 2012
Mucopolysaccharidosis (MPS) types I & II result from lysosomal accumulation of dermatan & heparan sulfate

Current treatment options:

**Hematopoietic stem cell transplantation (HSCT)**
- Severe MPS I only
- Significant mortality & morbidity risk

**Enzyme replacement therapy (ERT)**
- Frequent, hours-long infusions
- Does not cross blood-brain barrier
- Fails to treat some skeletal and cardiac valvular disease

Modified after Neufeld and Muenzer 2001 & Kowalewski 2012
Systemic delivery of ZFNs and transgene donor via AAV vectors for *in vivo* correction of monogenic disease

**In vivo genome editing**

- **AAV vectors**
- **ZFN1 and 2**
- **Therapeutic gene (IDUA or IDS)**
- **Strong albumin Promoter**
- **Albumin locus**
- **Therapeutic gene**
- **Homology arm**

Following *one-time dosing*, liver stably secretes therapeutic protein, which can *cross-correct* other tissues via circulation.
ZFN-mediated integration of an IDUA or IDS cDNA at the liver albumin locus results in secreted enzyme
Systemic delivery of ZFP therapeutics via AAV vectors in 
MPS I and MPS II mouse models

**AAV Vectors**

**Packaged into adeno-associated viral vectors (AAV)**

**One-time peripheral IV administration**

**MPS I mice (Ohmi et al.)**
- Male and female animals, single dose level: 7.5e13 vg/kg (assuming 20 g mouse)

**MPS II mice (Muenzer et al.)**
- Male animals only at three dose levels: 1.25, 2.5, 7.5e13 vg/kg (assuming 20 g mouse)

**Both models:**
- 1-2 month old mice at dosing
- Periodic plasma (activity) and urine (GAG) analysis
- 4 month cohort with neurological testing, full necropsy and histopathology

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**Albumin ZFN pair and corrective gene**

**ZFN1**

**ZFN2**

**Homology IDUA or IDS**

**Homology**
IDS is produced in the liver, secreted into plasma, & taken up by secondary tissues, with significant GAG reduction in **MPS II mice**

- **IDS activity** (nmol/hr/mg)
- **Total GAG Levels** (µg GAG/mg protein)
- **Plasma**

**Tissues** (4 months)

**Days post-injection**

- **ZFN+Donor vs MPS I Untreated**
  - P-values: *p<0.01; #p<0.05
Mass spectrometry confirms significant reduction of dermatan sulfate in the brains of **MPS I** and **MPS II** mice

*Dermatan Sulfate*

- **MPS I**
  - Wild type, Untreated
  - MPS I male, Untreated
  - MPS I female, Untreated
  - MPS I male, ZFN+Donor
  - MPS I female, ZFN+Donor
  - MPS I, Donor Only

- **MPS II**
  - Wild type, Untreated
  - MPS II, Untreated
  - MPS II, ZFN+Donor Low
  - MPS II, ZFN+Donor Mid
  - MPS II, ZFN+Donor High
  - MPS II, Donor Only

*Heparan Sulfate*

- **MPS I**
  - Wild type, Untreated
  - MPS I male, Untreated
  - MPS I female, Untreated
  - MPS I male, ZFN+Donor
  - MPS I female, ZFN+Donor
  - MPS I, Donor Only

- **MPS II**
  - Wild type, Untreated
  - MPS II, Untreated
  - MPS II, ZFN+Donor Low
  - MPS II, ZFN+Donor Mid
  - MPS II, ZFN+Donor High
  - MPS II, Donor Only

**P-values:** *p<0.05**

**LLOQ = 0.005 µg/mg**
Reduced cellular vacuolation in treated **MPS II** animals (4 months post-dosing)
Reduced cellular vacuolation in treated MPS I & MPS II animals (4 months post-dosing)

Reduced levels of vacuolation were also detected in treated MPS I & MPS II mice in:

- Liver (Kupffer cells)
- Spleen, Kidney & GI tract
- Heart & Lung
- Pituitary & Parathyroid glands
- Bone marrow, Thymus & Lymph nodes
- Bone (sternum) & Femoral-tibial joint
- Spinal cord (neuronal, glial cells; MPS I only)
ZFN+Donor treated MPS I and MPS II mice show significant preservation of cognitive function in Barnes maze.

**MPS I, Grouped**
- Male mice
- ZFN+Donor vs MPS Untreated
- *p<0.05, **p<0.001

**MPS II, Grouped**
- MPS II, High Dose
- Wild type

**MPS II, Individual mice, Day 6**
- Wild type
- MPS II
- MPS II, High Dose

Mice analyzed 4 months post-dosing.
hIDS produced from the human albumin locus is taken-up in an M6P-dependent manner *in vitro*

**Human Albumin ZFN pair and hIDS donor**

**Human Hepatoma HepG2 cells**

**HepG2 subclones producing hIDS**

**Secreted hIDS**

**IDS uptake in target cells**

- **Control media**
- **IDS supernatant**

**Graph**

- **Control**
  - IDS activity (nmol/hr/mg)
  - 0 200 400 600 800

- **Mannose-6-Phosphate**
  - **M6P receptor**
  - **Secreted hIDS**
  - **Lysosomal hIDS**
hIDS produced from the human albumin locus is taken-up in an M6P-dependent manner *in vitro*
AAV-mediated delivery of Albumin ZFNs and hIDUA or hIDS Donor in MPS I & II mice led to:

- Supraphysiological expression of active enzyme in the liver
- Secretion into plasma and
- Uptake into secondary tissues, at levels sufficient for reduction in GAG biomarker and tissue vacuolation

- Significant cognitive benefits were observed at 4 months (Barnes Maze)
- Durable enzyme expression and GAG reduction over 4 months
- Additional histopathology analysis shows that treatment is well-tolerated
- *In vitro* studies using human reagents demonstrate that highly active and glycosylated IDUA & IDS produced from the human Albumin locus are taken-up in an M6P-dependent manner

Phase 1/2 clinical trials are currently open to evaluate *in vivo* genome editing of the Albumin locus for the treatment of MPS I & MPS II
In 2017, Sangamo is focused on enrolling four clinical trials including the first ever human *in vivo* genome editing studies.

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*In Vivo* Gene Therapy  
*In Vivo* Genome Editing
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