Development and Validation of Enzyme Replacement Therapy for GM1 Gangliosidosis

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Abstract β-Galactosidase-deficient mice accumulate GM1 gangliosides in cells and tissues and exhibit many of the same neuropathological symptoms of patients suffering from GM1 Gangliosidosis. Intravenous enzyme replacement therapy would ameliorate ganglioside storage levels and many of the somatic aspects of the disease, but the blood brain barrier prevents uptake of recombinant enzyme to the brain, and therefore neurological symptoms of the disease are difficult to treat. Intracerebroventricular is a safe and effective method of delivering enzyme directly to the brain. Restoring the missing lysosomal enzyme in the brain of patients with GM1 Gangliosidosis is a significant unmet need. Our studies are aimed at intracerebroventricular delivery of β-galactosidase directly to the brain of GM1 mice. Efficacy to reduce pathological GM1 gangliosides and safety will be assessed in mouse intracerebroventricular dosing studies. Dose-dependent biodistribution of β-galactosidase and effects on biochemical and histological pathology in the brain of these GM1 mice, together with neurological behavior will be evaluated. These results should provide the preclinical information needed to proceed towards a novel treatment of the disease in GM1 Gangliosidosis patients. This work is funded by the Cure GM1 Foundation.

TEGA Therapeutics

TEGA Therapeutics is company working with Cure GM1 Foundation to develop an enzyme replacement therapy for the treatment of GM1 Gangliosidosis

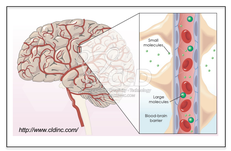
1. GM1 Gangliosidosis

- Autosomal recessive lysosomal storage disorder
- 1 in 100,000 - 300,000 children are born with the disease
- Unmet need: early death and progressive neurological disease
- Currently there is no treatment available

- Lysosomal storage disorder caused by the loss of β-galactosidase (*GLB1* gene)
- Resulting in toxic accumulation of complex sugars called GM1 gangliosides in the brain and systemic tissues
- Accumulation causes severe neurodegeneration, seizures and rapid developmental regression

2. Blood Brain Barrier

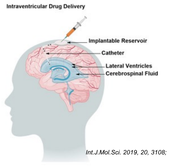
- Intravenous enzyme replacement therapy (ERT) exists in other lysosomal storage disorders and resolve somatic symptoms
- The blood brain barrier prevents uptake of recombinant enzyme to the brain
- Symptoms of the central nervous system in lysosomal storage diseases are difficult to treat



- Intracerebroventricular (ICV) is a safe and effective method of delivering enzyme directly to the brain
- Bypassing the blood brain barrier and delivering enzyme directly into the cerebral ventricles

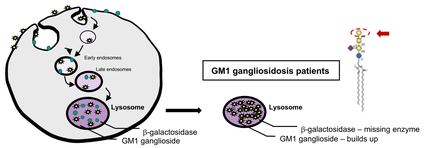
3. Approved ERT

- Brineura® – first FDA-approved safe and effective ICV ERT in pediatric patients with Batten/CLN2 disease (www.Brineura.com)
- Tralesinidase alfa – ongoing clinical trial underway using high dose of active enzyme ICV-delivered to MPS IIIB patients (https://clinicaltrials.gov/ct2/show/NCT02754076)
- This enzyme delivery approach should be safe and effective for treating children with GM1 gangliosidosis



4. Human β-Galactosidase

- Lysosomal enzyme removes β-linked galactose residues on ends of complex glycans
- GLB1 gene makes the enzyme that breaks down GM1 gangliosides inside lysosomes
- GM1 gangliosidosis patients are missing the GLB1 gene and cannot make active enzyme
- Resulting in toxic levels of GM1 gangliosides in the brain and spinal cord causing severe damage

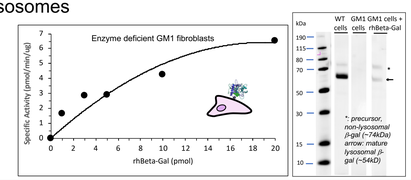


5. Clinical Disease Phenotypes

- **Infantile-onset is the severest form –**
  - Very low levels of residual β-galactosidase enzyme activity
  - Developmental delay in first year of life and rapid loss of motor skills and death by 2 - 4.5 years
- **Late-infantile, juvenile and adult-onset –**
  - Higher levels of residual β-galactosidase enzyme activity and longer survival
- Residual β-galactosidase activity range is very narrow among the different patients, ranging from 0-15% of normal control levels
- Minimal restoration of enzyme activity is needed to reduce GM1 Ganglioside build up and prevent disease progression

6. Enzyme Uptake

- *Can we deliver β-galactosidase to lysosomes and restore missing enzyme activity?*
- Mannose-6-phosphate receptors on cells target the enzyme to the lysosome
- GM1 patient fibroblasts deficient in enzyme treated with different concentrations of β-galactosidase
- Data showed β-galactosidase was taken up by cells and enzyme activity was restored
- Western blot analysis confirmed uptake of β-galactosidase into lysosomes



7. GM1 Gangliosidosis Mice

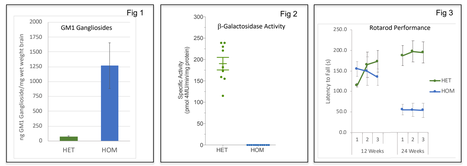
- Jackson Lab partnered with Cure GM1 Foundation to generate a mouse model of GM1 gangliosidosis
- CRISPR/CAS9 generated mutant of *GLB1* gene carrying a 7-nucleotide deletion in exon 5 predicting null allele
- Mice lacking β-galactosidase show a reduction in β-galactosidase enzyme activity in the brain *(WORLD 2020 Poster Presentation)*
- Impaired motor coordination with a reduction in latency to fall from accelerating rotarod *(WORLD 2020 Poster Presentation)*
- GM1 ganglioside accumulation was **not** measured in the mouse brains

8. GM1 Ganglioside Analysis

- **Do β-galactosidase deficient mice accumulate GM1 gangliosides in the brain?**
- Collected brain tissues from GM1 Gangliosidosis mice
- GM1 gangliosides were extracted and isolated from tissue samples
- Developed an analytical method in-house using mass spectrometry for quantifying GM1 gangliosides

9. GM1 Gangliosides Levels

- Data showed mice lacking β-galactosidase accumulate GM1 Gangliosides in the brain (Fig. 1)
- No β-galactosidase enzyme activity in the brain (Fig. 2)
- Impaired motor coordination with a reduction in latency to fall from accelerating rotarod (Fig. 3)
- Mouse model shows characteristics of a model of GM1 gangliosidosis



10. ICV Enzyme Delivery

- Studies are underway delivering β-galactosidase ICV to GM1 Gangliosidosis mice
- Efficacy and safety will be assessed in enzyme dosing studies
- Effects on biochemical and histological pathology in the brain will be determined
- Motor coordination in these mice will be evaluated
- Preclinical studies are an important step towards advancing this novel treatment for GM1 Gangliosidosis in patients

11. Summary

- Mouse model of GM1 gangliosidosis successfully established
- Significant GM1 ganglioside accumulation in the brain
- No detectable β-galactosidase enzyme activity in the brain
- Resulting in impaired motor coordination in these GM1 mice
- Promising preliminary data for both biochemical and neurobehavioral endpoints
- Studies are underway to determine effect of β-galactosidase after intracerebroventricular delivery

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