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In vitro evaluation of recombinant enzyme N-acetyl-alpha-glucosaminidase obtained from Komagataella phaffii GS115

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Mutations in *NAGLU gene* cause the loss of enzyme activity of *N*-acetyl-alpha-glucosaminidase (NAGLU) leading the accumulation of partially degraded heparan sulfate (HS) into the lysosomes. NAGLu deficiency causes the mucopolysaccharidosis type IIIB (MPS IIIB) or Sanfilippo syndrome type B, which currently has not an approved therapy. Enzyme replacement therapy (ERT) has remained as a possible therapeutic alternative. Recombinant enzymes used for development of possible ERT have been obtained from different expression systems as mammalian cells and microorganisms. Recently our group produced the recombinant NAGLU enzyme (rhNAGLU) in the yeast *Komagataella phaffii* GS115 reached up specific activity values of 2.39 U/mg after affinity purification. To evaluate the effect of the rhNAGLU as a possible therapeutic alternative, in the present study we carried out assays of cellular uptake, mannose and mannose-6-phosphate receptor inhibition, lysosomal traffic, and lysosomal mass reduction in an in vitro model of MPS IIIB fibroblast. Our results show that in MPS IIIB fibroblast rhNAGLU is partially endocytated through mannose- and mannose-6-phosphate receptor-dependent pathways. Upon the incubation with 100 nM of rhNAGLU MPS IIIB reached an intracellular specific activity (45.3 U/mg) close to wild-type levels (50.2 U/mg). Besides, using confocal microscopy approach, we found the colocalization between rhNAGLU and lysosomal compartments, suggesting that rhNAGLU is sorted from membrane plasma up to the lysosome. In addition, a reduction in the lysosomal mass was observed by flow cytometry quantification and fluorescence images upon 24- and 48-h post-treatment, suggesting that rhNAGLU is able to decrease the lysosomal storage in the MPS IIIB fibroblasts. In conclusion, our results demonstrate that rhNAGLU obtained from *K. phaffii* is an active and functional enzyme that could be useful in the future development of ERT-based therapies for the treatment of MPS IIIB.

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Psychosine predicts age of onset in babies with Krabbe disease

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Krabbe disease is a neurodegenerative disorder characterized by galactocerebrosidase(GALC) enzyme deficiency which causes psychosine toxicity. The patient phenotype is classified based on age at symptom onset, as infantile(0-12 months), late-infantile(13-47 months), juvenile(4-18 years), or adult(18 years+). Rapid developmental regression followed by death during early childhood is seen in untreated patients with infantile and late-infantile forms. Hematopoietic stem cell transplantation(HSCT) is the only treatment which can alter disease progression if performed before symptom onset(Infantile) or when the patient is minimally symptomatic(Late-Infantile). Thus, newborn screening(NBS) is likely to be the most effective way to detect and treat high-risk patients. The biomarker, psychosine, is used in NBS to predict early-onset phenotype. In this study, dried blood spots(DBS) psychosine was measured in treated and untreated patients. A total of 131 patients who were diagnosed or identified as being at high-risk of developing disease, were included. DBS were collected during evaluations between August 2010 and September 2021. Results from positive NBS were available for 24 patients. All 24 patients had elevated psychosine compared with controls. Newborn psychosine levels were found to be associated with onset of disease. Some babies with the infantile-onset had newborn psychosine as low as 2-6 nmol/L. Longitudinal follow-up indicated that both progression of disease and HSCT resulted in decline of psychosine. Pre- and post-transplant psychosine showed decline in 33 patients who underwent HSCT with no significant difference compared to decline after natural progression of disease. This is the largest study to date showing the relationship of psychosine to clinical phenotype. Based on these findings, we provide recommendations for newborn screening psychosine interpretation in babies with lower cut-off level of 2-6 nmol/L which is below the current 10 nmol/L cut-off for predicting the infantile phenotype. Although, psychosine declines during both progression of disease and after HSCT, further research is needed to understand if there are differing mechanisms for each.

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First-in-human phase I/II clinical trial of hematopoietic stem and progenitor cell gene therapy for Hurler syndrome: Favorable safety profile and extensive metabolic correction

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Allogeneic hematopoietic stem cell transplantation (allo-HSCT) +/− pre−/peri-transplant enzyme replacement therapy is standard of care for Hurler syndrome (MPS-IH). However, cognitive and skeletal abnormalities progress over time after allo-HSCT, severely affecting patient's quality of life. We report the interim results of a first-in-human phase I/II trial (NCT03488394) of 8 MPS-IH patients treated with autologous hematopoietic stem and progenitor cells (HSPC) genetically modified to overexpress human IDUA and followed-up for a median of 2.1 years after gene therapy (GT). Patients (6 M, 2 F; median age at treatment 24 months) lacked a non-heterozygous-HLA-matched cord blood donor and displayed IQ/DQ > 70. Primary efficacy endpoint was blood IDUA activity up to supraphysiologic levels at 1y post-GT. Clearance of lysosomal storage material, skeletal and neurophysiological development were assessed as secondary and exploratory endpoints. Mean drug product $CD34^+$ cell dose was 20.9×10^6/kg with a median vector copy number of 2.2 per genome. All patients had rapid hematologic recovery with median neutrophil engraftment on day +20 and short periods of thrombocytopenia. The 5 patients positive for anti-IDUA antibodies before GT cleared them within 3 months after GT. The procedure was generally well tolerated. All patients showed sustained engraftment of gene-corrected cells with blood IDUA activity reaching supraphysiologic levels after GT in all patients, maintained at last follow-up. Urinary glycosaminoglycan (GAG)

excretion levels reduced to normal or near-normal values by 1-year post-GT. IDUA activity in cerebrospinal fluid (CSF) became detectable by month 3 post-GT in all subjects accompanied by progressive decrease in CSF GAG storage. With a median follow-up of 2.1 years, patients show stable cognitive and motor performance, reduced joint stiffness, improved or stable findings on brain and spine MRI and normal growth according to peers. HSPC-GT accomplishes extensive metabolic correction and initial clinical response with a favorable safety profile, highlighting its therapeutic potential for MPS IH treatment.

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Substrate reduction therapy for Pompe disease: Small molecule inhibition of glycogen synthase 1 in preclinical models

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Pompe disease is a glycogen storage disease caused by mutations in the enzyme acid alpha-glucosidase (GAA) resulting in pathological accumulation of glycogen. Glycogen can accumulate in virtually all tissues, but the primary pathology affects skeletal and cardiac muscle leading to progressive weakness and respiratory compromise. The current standard of care for Pompe patients is GAA enzyme replacement therapy (ERT), which can ameliorate cardiac manifestations but does not stop the progressive skeletal muscle atrophy in infantile- or late-onset patients. Thus, there remains an important unmet need for new approaches to treat Pompe disease. In mouse models of Pompe disease, reducing glycogen synthesis via genetic ablation of GYS1 attenuates glycogen accumulation and muscle pathology. Glycogen synthase 1 (GYS1) is the rate-limiting enzyme in muscle glycogen biosynthesis. We report the discovery and preclinical characterization of orally bioavailable small molecule inhibitors of GYS1. These molecules potently and selectively decrease glycogen synthesis in muscle in vitro and in vivo without inhibiting liver glycogen synthase (GYS2). In multiple species, GYS1 inhibition reduces accumulation of glycogen as measured directly in tissues and by circulating biomarkers. In a mouse model of Pompe, chronic GYS1 inhibition lowers muscle glycogen and improves cellular markers of muscle pathology including lysosomal function and autophagy. When combined with ERT in a mouse model of Pompe, GYS1 inhibition augments the glycogen reduction in skeletal muscle. Furthermore, in vivo studies to date in multiple preclinical species as well as analysis of humans with naturally low levels of muscle glycogen support the tolerability of reduced muscle glycogen levels in humans. These studies indicate that small molecule inhibitors of GYS1 can modulate muscle glycogen levels and support their further development as substrate reduction therapy for patients with Pompe disease.

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Evaluation of neurofilament light chain as a biomarker for mucopolysaccharidosis type IIIB

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Mucopolysaccharidosis type IIIB (MPS IIIB, Sanfilippo syndrome B) is a lysosomal disorder caused by an autosomal recessive inherited deficiency of the NAGLU enzyme leading to the accumulation of the glycosaminoglycan heparan sulfate. MPS IIIB is primarily neuropathic and is characterized by progressive neurological impairment including developmental delay, behavioral symptoms, and impaired cognition. The naturally occurring canine model of MPS IIIB recapitulates the disease markers of MPS IIIB including cytoplasmic vacuolation, inflammation, and neurodegeneration. Canines begin to show clinical signs of disease between 18 and 24 months of age with the onset of mild neurologic symptoms, that progress to severe cerebellar dysfunction. There is currently no treatment for MPS IIIB as well as a lack of reliable biomarkers to monitor CNS disease during the pre-clinical period or to evaluate therapeutic response. Therefore, we sought to determine if neurofilament light chain (NfL) could act as a disease-relevant biomarker in MPS IIIB. NfL is a structural protein of the neuronal cytoskeleton, is readily released during neuroaxonal injury, stable in biofluids, and has been shown to be elevated in blood and cerebrospinal fluid (CSF) in several neurodegenerative diseases. Serum and CSF were collected from unaffected and MPS IIIB-affected canines between 4 and 32 months of age and NfL was measured using the highly sensitive Quanterix SIMOA® Neurofilament Light Advantage Kit. We found an age-dependent increase in serum and CSF NfL in MPS IIIB-affected dogs. NfL concentration did not significantly change over time in unaffected dogs. These data indicate that NfL may be a disease-relevant biomarker for MPS IIIB. Further exploration of fluid biomarkers will be critical for evaluating disease progression prior to clinical presentation, as well as for monitoring treatment response during the development of therapies for the MPS disorders.

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Long-term hematopoietic stem cell lentiviral gene therapy rescues neuromuscular manifestations in preclinical study of Pompe disease mice

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Pompe disease is an inherited neuromuscular disorder caused by acid alpha-glucosidase (GAA) deficiency, leading to lysosomal glycogen accumulation in tissues resulting in muscle weakness. If untreated, the most severely affected patients typically succumb early in life due to cardiorespiratory failure. The standard of care is enzyme replacement therapy, which requires lifelong treatment, but