Poster # 106

M021: A Uniquely Glycosylated, Highly Phosphorylated Acid-Alpha Glucosidase Enzyme Replacement Therapy for the Treatment of Pompe Disease

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Abstract

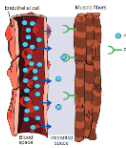
Pompe disease is a rare inherited metabolic disorder of defective lysosomal glycogen catabolism due to a deficiency in acid alpha-glucosidase (GAA). Alglucosidase alfa enzyme replacement therapy (ERT) using recombinant human GAA (rhGAA ERT) has provided irrefutable clinical benefits but clinical benefits have been limited primarily due to poor drug targeting of ERT to skeletal muscles. We have developed a novel rhGAA (M021) that was co-expressed with S1S3 phosphotransferase that greatly enhanced mannose 6-phosphate levels of rhGAA in the production cell line. As a result, M021 is produced with high amounts of bis-phosphorylated glycans (>50% of total oligosaccharides) and was shown to have very high binding affinity for the cation-independent M6P receptor (CI-MPR). Further, M021 has astonishingly low levels of neutral oligosaccharides (<5%). This unique oligosaccharide profile maximizes CI-MPR targeting while minimizing unproductive mannose receptor and asialoglycoprotein receptor clearance in the liver. This would likely translate into an optimal pharmacokinetics profile with better biodistribution to target muscles for more effective glycogen reduction. Our early results indicate that 20 mg/kg M021 was significantly more effective than 80 mg/kg alglucosidase alfa for reducing accumulated glycogen in muscles of *Gaa*KO mice. These promising results suggest that M021 warrant further development as a potential next-generation treatment for Pompe disease.

Pompe Disease

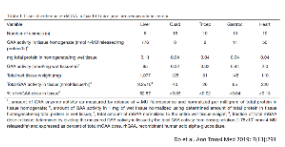
- Pompe disease is an inherited (genetic) disorder in which toxic levels of the complex sugar glycogen accumulates in cells
  - Infantile-onset Pompe disease has an onset age <12 months and affects the heart muscle (cardiomyopathy); muscle weakness, enlarged liver and heart, difficulty breathing and feeding occur
  - Late-onset Pompe disease has an onset age >12 months or onset age <12 months without cardiomyopathy; progressive muscle weakness, difficulty breathing, chronic pain, enlarged organs, and other symptoms occur
- Pompe is an ultrarare autosomal recessive disorder
- Pompe disease is caused by low levels or absence of acid alfa-glucosidase (GAA), an enzyme that normally breaks down glycogen in the lysosome, due to mutations in the GAA gene encoding GAA
- GAA deficiency results in glycogen accumulating in lysosomes in multiple tissues, particularly cardiac and skeletal muscle
- Treatments addressing GAA enzyme deficiency
  - Enzyme replacement therapy (ERT)

    Multiple investigational products in development, including gene therapy
- Cardiac and skeletal muscles are not well targeted by current SOC

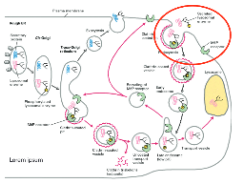
Pompe is a not a blood disease



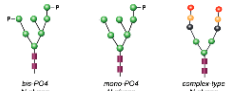
Less than 1% of the administered dose reaches most severely impacted tissues



CI-MPR is the Main Receptor Responsible for Targeting Lysosomal Proteins to Lysosomes



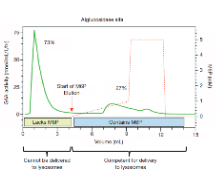
Not all phosphorylated glycans are equal.
Bis-phosphorylated glycans have ~3,000x greater affinity for the CI-MPR than Mono-phosphorylated glycans



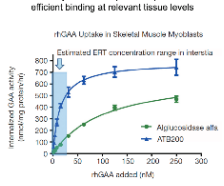
Ligand	Binding affinity (Apparent K_D; nM)*
bis-phosphorylated N-glycan	2
mono-phosphorylated N-glycan	>6900
complex type N-glycan	No binding

Alglucosidase Alfa is a Poor Ligand for the CI-MPR

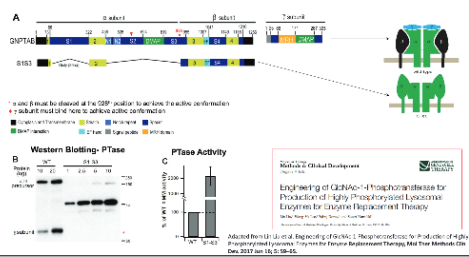
Only about 1 in 4 GAA molecules can bind the CI-MPR



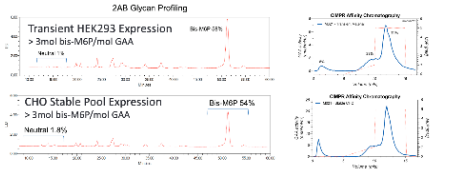
Affinity for the receptor is to poor for efficient binding at relevant tissue levels



S1S3 Truncated PTase has ~20X Specific Activity to Enable Better Phosphorylation of Lysosomal Enzymes

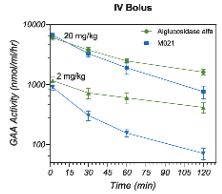


M021: Pompe Enzyme Replacement Therapy with Optimized Oligosaccharides



- Historically, endogenous and rhGAA are poorly phosphorylated
- Regardless of the expression system, rhGAA is highly phosphorylated when co-expressed with S1S3 GlcNAc-Phosphotransferase
- >85% of the N-linked glycans found on M021 are phosphorylated. With >50% being bis-phosphorylated resulting in very high affinity (<5 nM KD) for the CI-MPR that ensure efficient cellular uptake
- Non-productive clearance via mannose receptor and asialyloglycoprotein receptor should be minimal due to low levels of neutral oligosaccharides.

Serum Half-life is Reflective of Oligosaccahride Differences Between M021 and Alglucosidase alfa

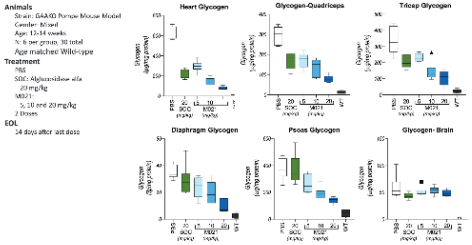


20 mg/kg	Alglucosidase alfa	M021
Half-Life (min)	50	30
AUC (nmol/ml/hr)	353,539	297,316

2 mg/kg	Alglucosidase alfa	M021
Half-Life (min)	60	25
AUC (nmol/ml/hr)	76,659	30,514

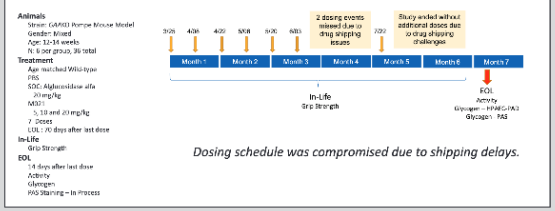
- M021 contains mainly phosphorylated high mannose glycans which target the CI-MPR pathway for productive cellular uptake by skeletal and cardiac tissue
- Alglucosidase alfa is predominate composed of complex type glycans found on plasma proteins which are not rapidly cleared from the blood

M021 is significantly better than SOC for reducing accumulated glycogen in short term efficacy studies



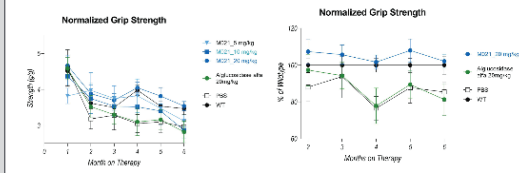
In the Cardiac and Skeletal Muscle, M021 is significantly better at reducing glycogen. Unfortunately the brain glycogen was not reduced.

M021: Long Term Efficacy Evaluated



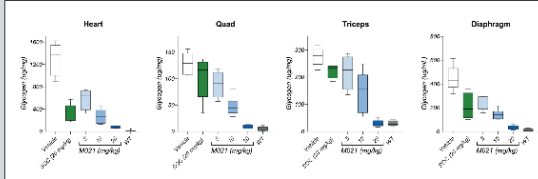
Dosing schedule was compromised due to shipping delays.

M021: Able to Maintain Wild-type Level Grip Strength during Treatment @ 20 mg/kg. SOC was Ineffective at the Same Dose



- Grip Strength data collections was variable across time, however, normalizing to wild-type grip strength helps to evaluate effect over time
- Animals on 20 mg/kg had grip strength comparable to wild-type animals

M021 Normalized Glycogen Levels to WT Levels in Skeletal Muscles in Long-term Study



- M021 at 20 mg/kg glycogen levels were normalized to near wild-type levels even with the delay/missed in dosing
- Despite missing treatment for multiple weeks, the *in vivo* impact of M021 for normalizing glycogen was maintained

Conclusions

- S1S3 PTase efficiently and reliably phosphorylates soluble lysosomal enzymes including those that are typically poorly phosphorylated like GAA
- On average, there is >3 mol of M6P per mole of M021, leading to high affinity binding to the CI-MPR receptor (2-3 nM, data not shown)
- Pre-clincial studies of M021 in the Pompe mouse model demonstrated robust efficacy as measured by in-life grip strength and glycogen reduction.
- These promising results suggest that M021 warrants further development as a potential next-generation treatment for Pompe disease

Acknowledgments

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