Poster #077
Wednesday
3-7 pm

What is in Store for Pompe Disease Therapy?

A More Potent ERT that Rapidly Normalizes Glycogen and Muscle Function Which Can Be Subsequently Maintained with Less Frequent Monthly Dosing

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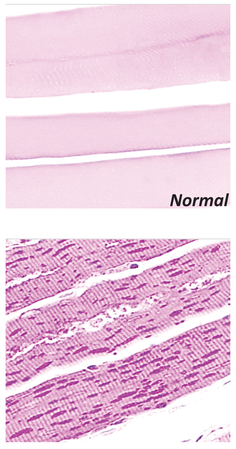
Pompe Disease

- Pompe disease is an inherited (genetic) disorder in which toxic levels of the large complex sugar, glycogen, accumulates in muscle 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 rare autosomal recessive disorder
- Pompe disease is caused by reduced or absent acid alpha-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 muscles
- Recombinant human GAA enzyme replacement therapy (rhGAA ERT) is the current approved treatment for supplementing GAA enzyme deficiency
  - Alglucosidase alfa ERT (Sanofi) is the current standard of care (SoC) for Pompe
  - SoC cannot slow disease progression and thus, more effective ERTs are required for unmet medical needs
  - Next generation ERTs Avalglucosidase alfa (Sanofi) & Cipaglucosidase alfa (Amicus) were shown to be non-inferior to SoC in clinical trials
  - Development of a more effective treatment is still needed

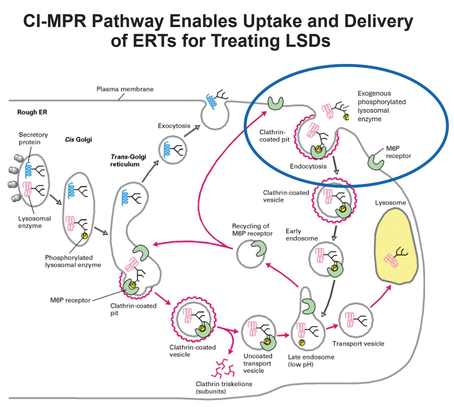
PAS Glycogen Staining of Skeletal Muscles



Leslie et al. Pompe disease. GeneRev 2017
Kohler et al. Neurhother 2018
EU lex defines ultra-rare disease as affecting 1 in 50,000 [OJ 27/5/2014; L 158/1]

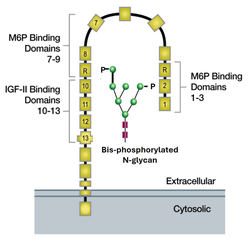
Natural M6P Receptor Pathway Enables Phosphorylated Exogenous Lysosomal Enzymes Cellular Uptake for Treatment of Lysosomal Disease

CI-MPR Pathway Enables Uptake and Delivery of ERTs for Treating LSDs

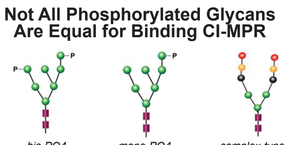


Lodish H, Berk A, Zipursky SL, et al. Molecular Cell Biology. 4th edition. New York: W. H. Freeman; 2000. Section 17.7

CI-MPR Structure



Not All Phosphorylated Glycans Are Equal for Binding CI-MPR

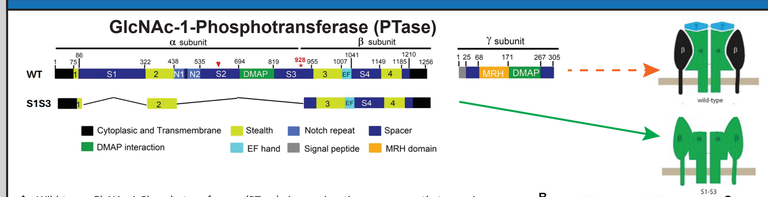


Ligand	Binding affinity (Apparent K_D; nM)*
bis-phosphorylated N-glycan	2
mono-phosphorylated N-glycan	>6000
complex type N-glycan	No binding

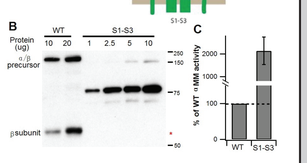
Adapted from Tong et al., 1989

S1S3 Variant Has Key Attributes That Enable Its Use for Development of Best-In-Class Recombinant Enzymes and Gene Therapies

GlcNAc-1-Phosphotransferase (PTase)

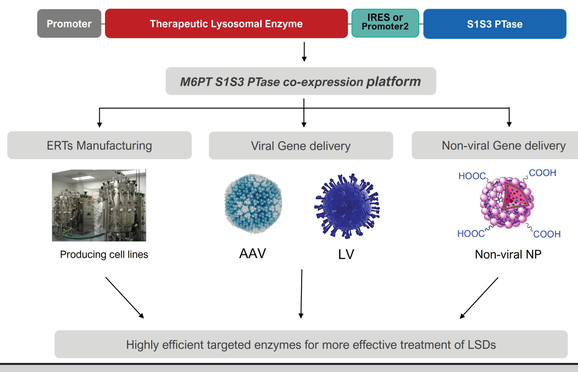


- Wild-type GlcNAc-1-Phosphotransferase (PTase) is an inactive zymogen that requires proteolytic cleavage at amino acid residue 928 (red box) to achieve its active conformation
- No known way to increase proteolytic cleavage and enzyme activation within cells for increasing enzyme activity for improving phosphorylation
- S1S3 PTase is a truncated, pre-activated variant with significant advantages:
  - Has proper conformation without need for γ-subunit
  - Has 20-fold higher specific activity
  - Enables efficient phosphorylation of soluble lysosomal enzymes
  - Small gene size enables its incorporation into expression constructs for S1S3 PTase co-expression with therapeutic enzymes within cells for ERT and gene therapy applications



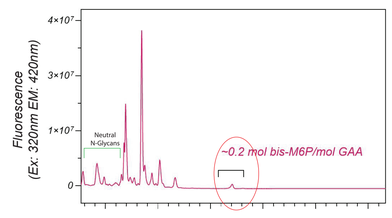
Lin Liu et al. Engineering of GlcNAc-1-Phosphotransferase for Production of Highly Phosphorylated Lysosomal Enzymes for Enzyme Replacement Therapy, Mol Ther Methods Clin Dev. 2017 Jun 16; 5: 59–65

S1S3 PTase Co-Expression Platform Ensures Production of Highly Phosphorylated Lysosomal Enzymes for ERTs and GTx



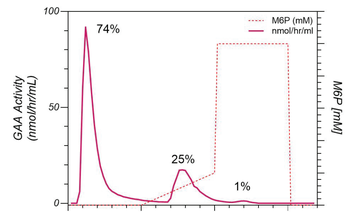
M021 is a Unique rhGAA that Contains High Levels of Bis-Phosphorylated N-Glyans When Produced With S1S3 PTase Co-Expression

Oligosaccharide Mapping

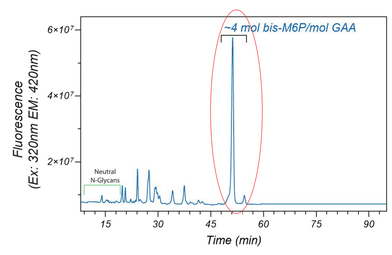


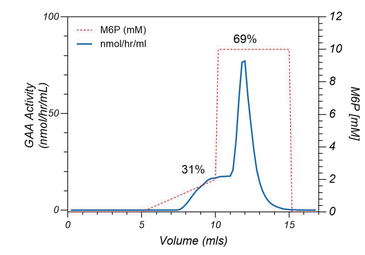
CI-MPR Affinity Chromatography

Alglucosidase alfa



M021 (rhGAA co-expressed with S1S3 PTase)

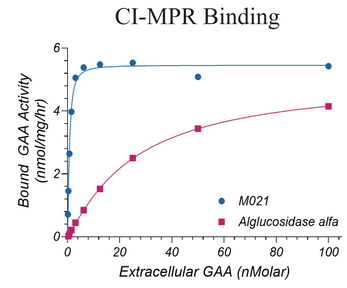




- Alglucoside alfa is composed of 70% complex-type N-glycans and ~30% high mannose-type N-glycans. Majority of high mannose glycans are not phosphorylated which severely limits CI-MPR binding leading to low cellular uptake and delivery to the lysosome.
- M021 is naturally produced in CHO cells with an average of ~4 moles of bis-phosphorylated high-mannose N-glycans per mole of GAA, leading to high-affinity binding to the CI-MPR.
- M021 has very low amounts of neutral N-glycans, which avoids clearance via mannose and asialyoglycoprotein receptors found in liver, spleen and other tissues.

M021 has Better CI-MPR Binding Enabling Greater Cellular Uptake than Alglucosidase alfa

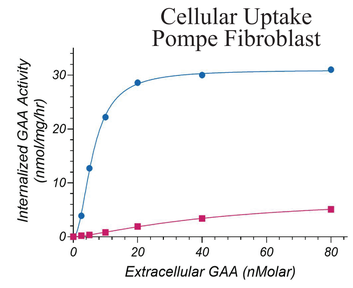
CI-MPR Binding



	M021	Alglu
Bmax (nmol/ml/hr)	5.5	ND
Kd (nM)	0.8	ND

ND: Not Determined since Bmax was not acheived

Cellular Uptake Pompe Fibroblast

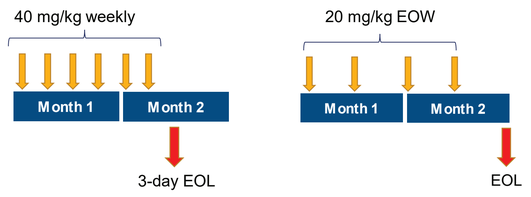


	M021	Alglu
Bmax (nmol/mg/hr)	31	ND
Kuptake (nM)	6	ND

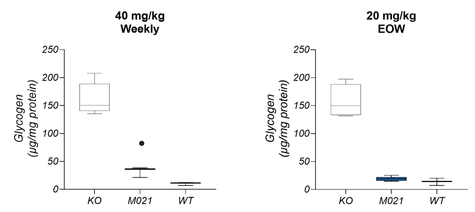
ND: Not Determined since Bmax was not acheived

M021 Can Reduce Accumulated Glycogen to Near Wild-type Levels

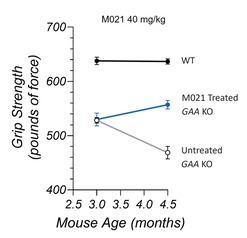
Animals
Strain: *Gaa*KO Pompe Mice
Gender: Mixed
4M or 11M at start
N: 4-6 per group
Age matched Wild-type
Treatment
M021
40 mg/kg for 6 doses, Weekly
20 mg/kg for 4 doses, EOW
EOL
3 or 14 days after last dose



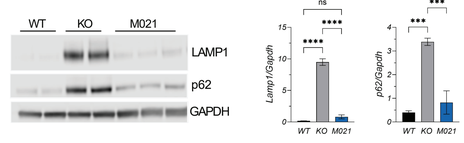
Gastrocnemius Glycogen



Grip Strength



Cellular Pathology Markers



Note: Increased levels of LAMP1 and p62 are established markers of defective autophagy and altered vesicular trafficking

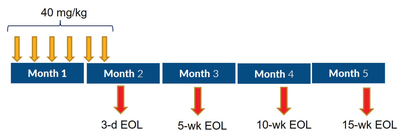
Lim et al. Pros and cons of different ways to address dysfunctional autophagy in Pompe disease, Ann Transl Med. 2019;7(13):279

*Statistical significance was determined using unpaired t-tests where NS Not Significant, ***P<0.001, ****P<0.0001*

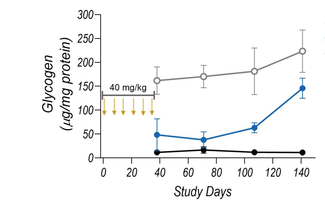
- A better targeted ERT and time, are needed for effective glycogen clearance
- With rapid normalization of glycogen there is a correlative improvement in muscle function (grip strength)

After Normalization of Glycogen, Glycogen Reaccumulation is Slow Following Cessation of Therapy

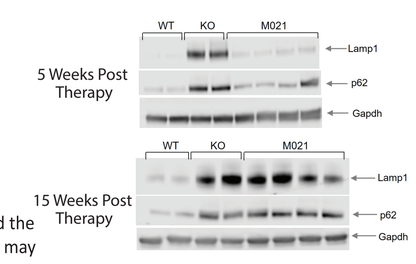
Animals
Strain: *Gaa*KO Pompe Mice
Gender: Mixed
12-16 Weeks
N: 5- 6 per group
Age matched Wild-type
Treatment
M021- 40 mg/kg
6 weekly doses
EOL
3D, 5W, 10W, or 15W



Gastrocnemius Glycogen



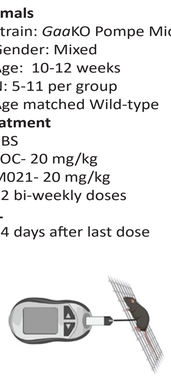
Gastrocnemius Pathology Markers

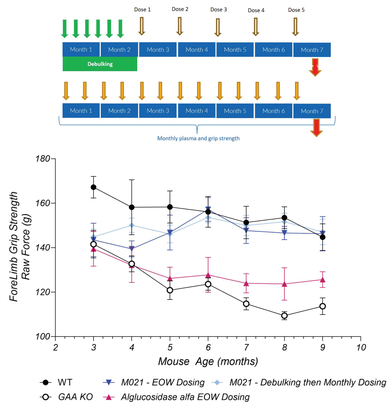


- M021 can rapidly clear accumulated glycogen and the glycogen re-accumulation is relatively slow which may enable less frequent monthly maintenance dosing

M021 Rapidly Normalizes Functional Grip Strength After Debulking Which Can Be Maintained With Less Frequent Monthly Maintenance Dosing

Animals
Strain: *Gaa*KO Pompe Mice
Gender: Mixed
Age: 10-12 weeks
N: 5-11 per group
Age matched Wild-type
Treatment
PBS
SOC- 20 mg/kg
M021- 20 mg/kg
12 bi-weekly doses
EOL
14 days after last dose

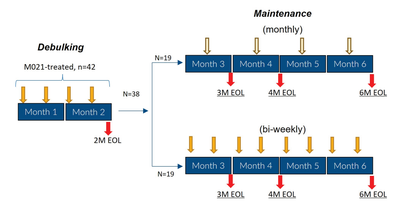




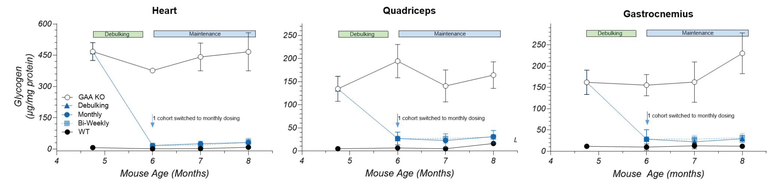
- Grip strength was significantly improved with M021 ERT by 2 months and indistinguishable from wild-type mice by 3 months.
- Improved grip strength maintained using monthly M021 dosing.

M021 Rapidly Normalizes Glycogen, Reverses Cellular Dysfunction and Enables Less Frequent Monthly Maintenance Dosing Without Aggressive Debulking

Animals
Strain: *Gaa*KO Pompe Mice
Gender: Mixed
4M at start
N: 4-5 per group
Age matched Wild-type
Treatment
M021- 20 mg/kg
All animals received bi-weekly dosing for 4 doses
Then one cohort switched to **monthly** dosing
The other cohort remained on **bi-weekly** dosing
EOL
14 days after last dose



Tissue Glycogen



- Four bi-weekly administrations of M021 (20 mg/kg) substantially reduced accumulated glycogen to near wildtype levels.
- Following debulking, near normal glycogen levels can be maintained using less frequent monthly dosing.
- Normalization of glycogen correlated with reversal of cellular dysfnction as evidenced by reduction of LAMP1 & p62.

Conclusions

- M021 is a unique rhGAA ERT with ideal N-glycosylation profile: naturally high levels of bis-phosphorylated N-glycans to enable efficient CI-MPR binding and cellular uptake; low levels of neutral glycans to minimize non-productive clearance in liver, spleen, etc.
- M021 rapidly clears glycogen, reverses cellular pathology and normalizes functional grip strength in treated Pompe mice.
- These data suggest that M021 is a much more potent rhGAA ERT with potential for less frequent dosing.

Acknowledgements

- Marshall's Mountain for sponsoring visiting researcher, Emilee Statzer.
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