Poster #115
Wednesday 3-7 pm

M021: A Novel Drug Candidate for Pompe Disease
Development of a Best-In-Class ERT for Pompe Disease

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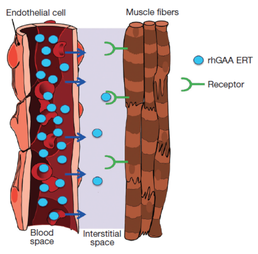
Pompe Disease

- Pompe disease is an inherited disorder in which pathological 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 a rare autosomal recessive disorder
- Pompe disease is caused by low levels or absence of acid alpha-glucosidase (GAA), an enzyme that normally breaks down glycogen in the lysosome, due to mutations in the gene encoding GAA
- GAA deficiency leads to lysosomal glycogen accumulating 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
- Skeletal muscles are not well targeted by current SOC

Pompe is not a Hematological Disorder



Less than 1% of the adminstered dose reaches the most severly impacted tissue

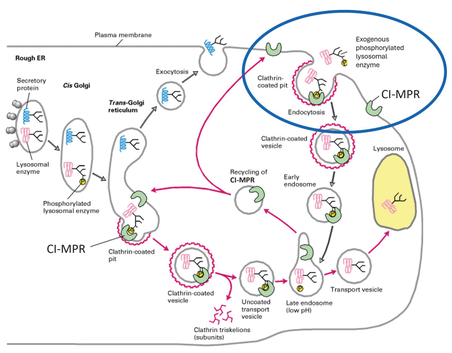
Table 1 Tissue distribution of rhGAA in Gaa KO mice post-intravenous administration

Variable	Liver	Quad	Tricep	Gastroc	Heart
Number of animals (n)	6	16	10	10	10
GAA activity in tissue homogenate (nmol 4-MU released/mg protein/hr)[a]	776	8	8	11	50
mg total protein in homogenate/mg wet tissue	0.11	0.04	0.04	0.04	0.04
GAA activity (nmol/mg wet tissue/hr)[b]	85	0.32	0.32	0.44	2.0
Total wet tissue weight (mg)	1,077	125	81	148	110
Total GAA activity in tissue (nmol/tissue/hr)[c]	9.2×10^4	40	26	65	220
% of rhGAA dose in tissue[d]	52.57	<0.03	<0.02	<0.04	<0.13

[a], amount of GAA enzyme activity as measured by release of 4-MU fluorescence and normalized per milligram of total protein in tissue homogenate; [b], amount of GAA activity in 1 mg of wet tissue normalized using determined amount of total protein in tissue homogenate/mg total protein in wet tissue; [c], total amount of rhGAA normalized to the entire wet tissue weight; [d], fraction of total rhGAA dose in tissue determined by dividing the measured GAA activity in tissue by the total GAA activity from dosing solution (1.75×10^7 nmol 4-MU released/hr) and expressed as percent of total rhGAA dose. rhGAA, recombinant human acid alpha-glucosidase.

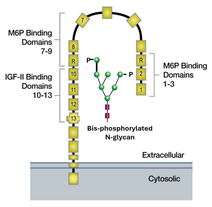
Natural M6P Receptor Pathway Enables Phosphorylated Exogenous Lysosomal Enzymes Cellular Uptake for Treatment of Lysosomal Disease

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

Structure of CI-MPR



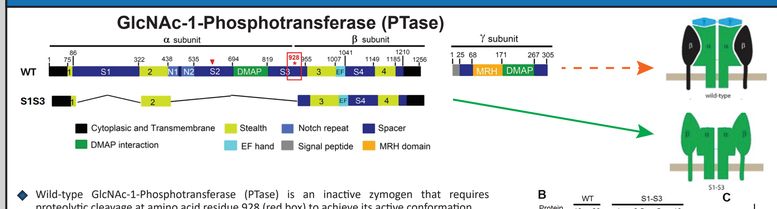
Not All Phosphorylated Glycans Are Equal for Binding CI-MPR

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

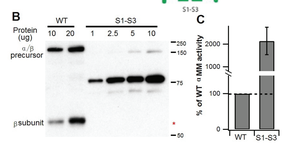
Adapted from Tong et al., 1989

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

GlcNAc-1-Phosphotransferase (PTase)

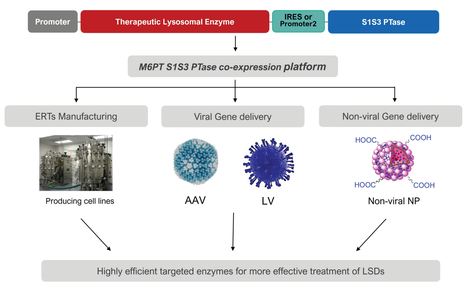


- Wild-type GlcNAc-1-Phosphotransferase (PTase) is an inactive zymogen that requires proteolytic cleavage at amino acid residue 928 (red box) to achieve its active conformation
- No known way to increase proteolytic cleavage and enzyme activation within cells for increasing enzyme activity for improving 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

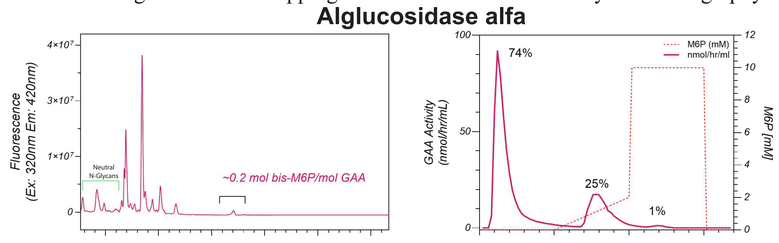
S1S3 PTase Co-Expression Platform Ensures Production of Highly Phosphorylated Lysosomal Enzymes for ERTs and GTx



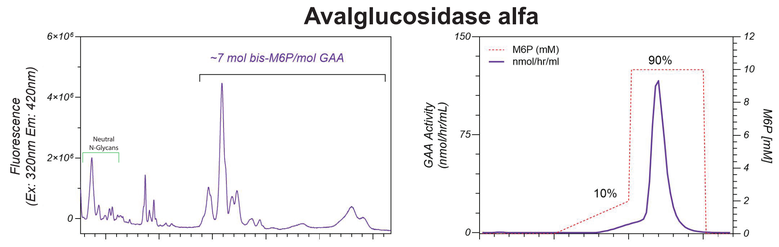
M021 is Naturally Produced with High Levels of Bis-Phosphorylated N-Glyans by Co-Expression with S1S3 PTase

Oligosaccharide Mapping | CI-MPR Affinity Chromatography

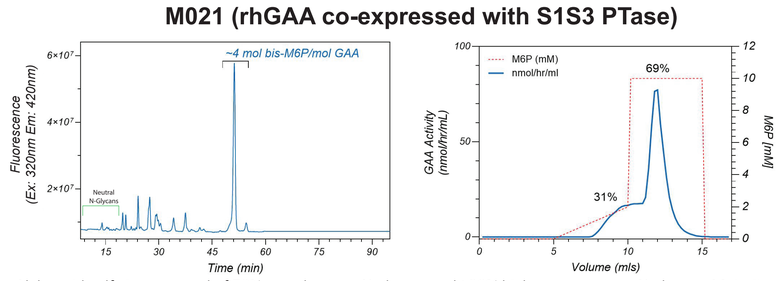
Alglucosidase alfa



Avalglucosidase alfa

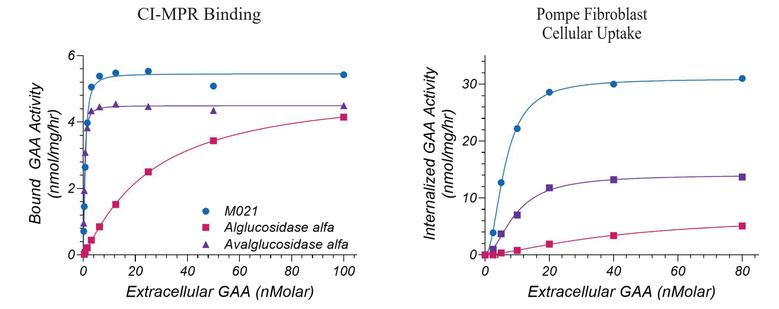


M021 (rhGAA co-expressed with S1S3 PTase)



- 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.
- Avalglucosidase alfa is produced by chemical coupling of synthetic bis-phosphorylated N-glycans onto modified sialic acids of preexisting N-glycans of alglucosidase alfa. The resultant formed oxime bond is very stable and unhydrolyzable within cells.
- On average, approximately 7 novel bis-phosphorylated glycans are added (Anding A et al. J Pharmacol Exp Ther. 2023;387(2):188-203.) which leads to much improved affinity for CI-MPR.
- 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 and Avalglucosidase alfa



	M021	Alglu	Aval
Bmax (nmol/ml/hr)	5.5	ND	4.5
Kd (nM)	0.8	ND	0.5

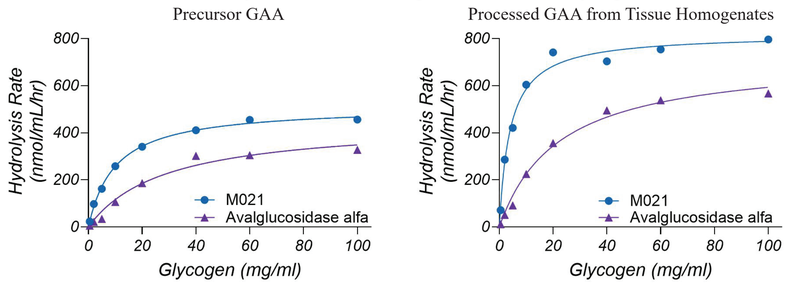
ND: Not Determined since Bmax was not achieved

	M021	Alglu	Aval
Bmax (nmol/mg/hr)	31	ND	14
Kuptake (nM)	6	ND	9

ND: Not Determined since Bmax was not achieved

Chemical Coupling of Synthetic M6P-bearing Glycans Alter Avalglucosidase Alfa's Ability to Effectively Hydrolyze Glycogen

Enzyme Kinetics using Bovine Liver Glycogen



	M021	Aval
Vmax (nmol/ml/hr)	~456	~327
Km (mg/mL)	10	28

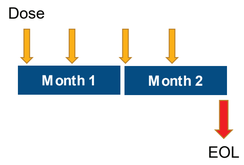
	Mature M021	Processed Aval
Vmax (nmol/ml/hr)	~800	ND
Km (mg/mL)	4.0	ND

ND: Not Determined since Vmax was not achieved

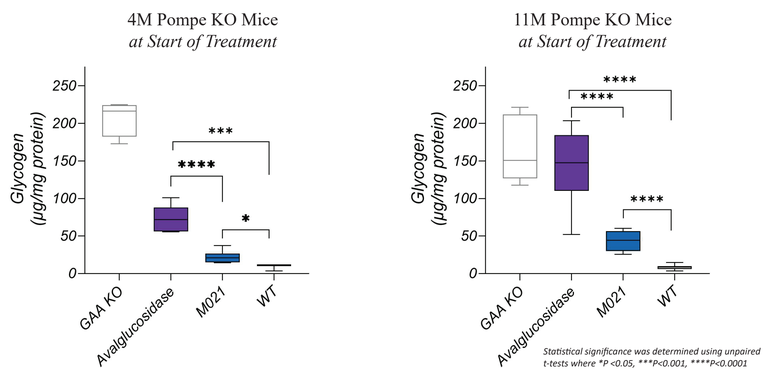
- Avalglucosidase has normal kinetics for small substrates such as 4MU-α-glucoside but has significantly lower kinetics for large substrates like glycogen, particularly in older mice where glycogen in more branched and complicated.
- The formed oxime bonds cannot be hydrolyzed in cells, thereby impeding GAA processing and activation which alters enzyme kinetics towards glycogen.

M021 is Significantly Better than Avalglucosidase for Reducing Accumulated Glycogen in Young and Old Pompe Mice

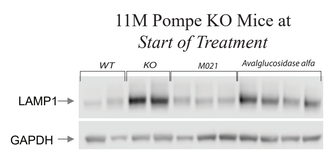
Animals
Strain: *Gaa*KO Pompe Mice
Gender: Mixed
4M or 11M at start
N: 5- 11 per group
Age matched Wild-type
Treatment
M021- 20 mg/kg
Avalglucosidase alfa - 20 mg/kg
4 doses, every other week
EOL
14 days after last dose



Quadriceps Glycogen



Lysosomal Marker (LAMP1) Evaluation

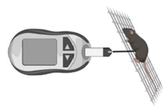


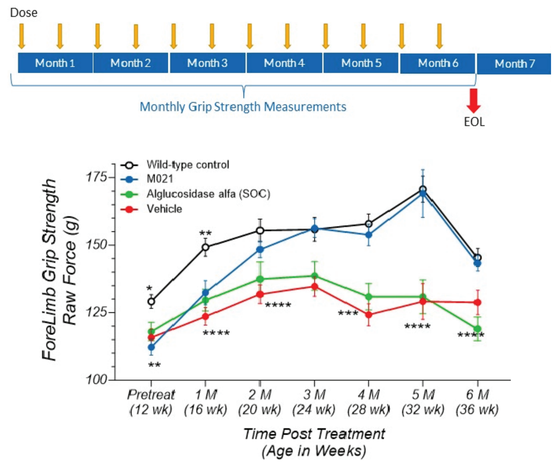
Lim et al. Pros and cons of different ways to address dysfunctional autophagy in Pompe disease, Ann Transl Med. 2019;7(13):279

- M021 effectively reduced glycogen in young and old Pompe mice.
- Avalglucosidase was able to reduce glycogen in the young Pompe mice albeit not as well as M021.
- Avalglucosidase had minimal glycogen reduction in older mice, likely due to the diminished ability to hydrolyze more branched and complex glycogen substrate in older mice (consistent with kinetic data).
- M021 reversed cellular pathology as evidenced by reduced LAMP1 that is comparable to WT mice.
- Avalglucosidase was much less effective in reducing LAMP1 compared to M021

M021 Rapidly Normalized Muscle Grip Strength in Treated Pompe Mice to That of Wild-Type while Alglucosidase alfa Did Not Normalize Grip Strength

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 phenotype was significantly improved by M021 ERT by 2 months and indistinguishable from wild-type mice by 3 months.
- SOC was not able to improve grip strength under identical experimental conditions.

Conclusions

- S1S3 PTase co-expression efficiently and reliably produces naturally phosphorylated N-glycan soluble lysosomal enzymes including those that are typically poorly phosphorylated like GAA.
- On average, there is >4 mol of bis-M6P N-glycans per mole of M021, resulting in high affinity binding to the CI-MPR enabling the highest cellular uptake.
- In head-to-head studies, M021 is significantly better than alglucosidase alfa and avalglucosidase alfa for reducing accumulated glycogen, even in old Pompe mice. Avalglucosidase alfa was not able to reduce glycogen in old mice under identical conditions.
- M021 was also able to normalized grip strength to that of wild type mice after 3 months of treatment- a feat that had not been accomplished with previous ERTs.

Acknowledgements

- Marshall's Mountain for sponsoring visiting researcher, Emilee Statzer.
- House Foundation for sponsoring critical pre-clinical animal studies.
- Sanofi for suppling alglucosidase alfa and avalglucosidase alfa.