M021 (rhGAA) Has an Optimal Oligosaccharide Composition That Allows for Efficient Reduction of Skeletal Muscle Glycogen and Recovery of Grip Strength in a Pompe Mouse Model

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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 an ultra-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.

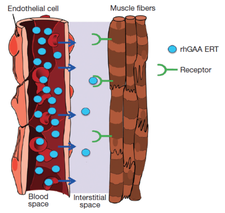
GAA deficiency leads to lysosomal glycogen accumulating in multiple tissues, particularly cardiac and skeletal muscle.

Treatments addressing GAA enzyme deficiency:

- Enzyme replacement therapy (ERT)
- Multiple investigational products in development, including gene therapy

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

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.

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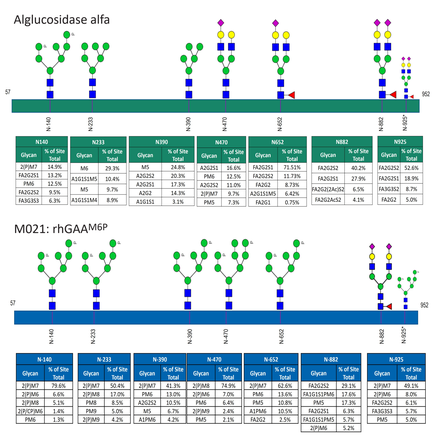
M021 is a Unique rhGAA that contains High Levels of Bis-Phosphorylated N-Glyans when Produced with S1S3 PTase Co-Expression

Site-Specific Glycopeptide Mapping LC-MS/MS



N-140 Glycan	% of Site Total
2(P)M7	14.9%
FA2G2S1	13.2%
PM6	12.5%
FA2G2S2	9.5%
FA3G3S3	6.3%

N-233 Glycan	% of Site Total
M6	29.3%
A1G1S1M5	10.4%
M5	9.7%
A1G1S1M4	8.9%

N-390 Glycan	% of Site Total
M5	24.8%
A2G2S2	20.3%
A2G2S1	17.3%
A2G2	14.3%
A1G1S1	3.1%

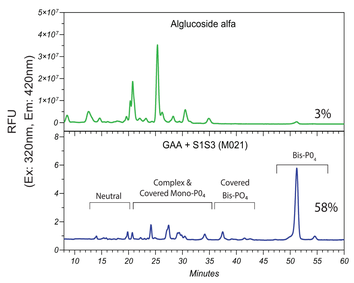
N-470 Glycan	% of Site Total
A2G2S1	16.6%
PM6	12.5%
A2G2S2	11.0%
2(P)M7	9.7%
PM5	7.3%

N-652 Glycan	% of Site Total
FA2G2S1	71.51%
FA2G2S2	11.73%
FA2G2	8.73%
A2G1S1M5	6.42%
FA2G1	0.75%

N-882 Glycan	% of Site Total
FA2G2S2	40.2%
FA2G2S1	27.9%
FA2G2Gal2GlcNAc2	6.5%
FA2G2Ac4S2	4.1%

N-925 Glycan	% of Site Total
FA2G2S2	52.6%
FA2G2S1	18.9%
FA3G3S2	8.7%
FA2G2	5.0%

Fluorescently Labeled Glycan Chromatography



N-140 Glycan	% of Site Total
2(P)M7	79.6%
2(P)M6	6.6%
2(P)M8	5.1%
2(P)G(P)M6	1.4%
PM6	1.3%

N-233 Glycan	% of Site Total
2(P)M7	50.4%
2(P)M8	17.0%
PM8	8.5%
PM9	5.0%
2(P)M9	4.2%

N-390 Glycan	% of Site Total
2(P)M7	41.3%
PM6	13.0%
A2G2S2	10.5%
M5	6.7%
A1PM6	4.2%

N-470 Glycan	% of Site Total
2(P)M8	74.9%
2(P)M6	7.0%
PM6	6.4%
PM5	2.4%
A1PM6	2.1%

N-652 Glycan	% of Site Total
2(P)M7	62.6%
PM6	13.6%
PM5	10.8%
A1PM6	10.5%
FA2G2	2.5%

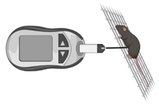
N-882 Glycan	% of Site Total
FA2G2S2	29.1%
FA1G1S1PM6	17.6%
PM5	17.3%
FA2G2S1	6.3%
FA1G1S1PM5	5.7%
2(P)M6	5.2%

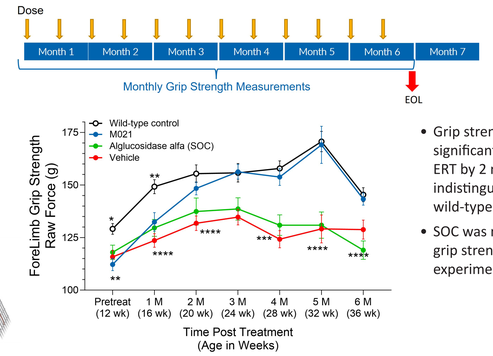
N-925 Glycan	% of Site Total
2(P)M7	49.1%
2(P)M6	8.0%
FA2G2S2	6.1%
FA3G3S3	5.7%
PM5	5.0%

- Alglucoside alfa is composed of 70% complex-type N-glycans, whereas M021 is composed of mainly phosphorylated high mannose glycans.
- On average, there are >3 moles of M6P per mole of M021, leading to high-affinity binding to the CI-MPR.

M021 Rapidly Normalized Muscle Grip Strength in Treated Pompe Mice to that of Wild-type while SOC did not

Animals
Strain: *GAA*^{KO} Pompe Mice
Gender: Mixed
Age: 10-12 weeks
N: 5-11 per group
Age matched WT controls
Treatment:
PBS
SOC – 20 mg/kg
M021 - 20 mg/kg
Age matched WT
12 bi-weekly doses
EOL
14 days after last dose

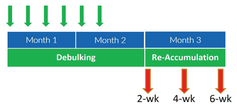


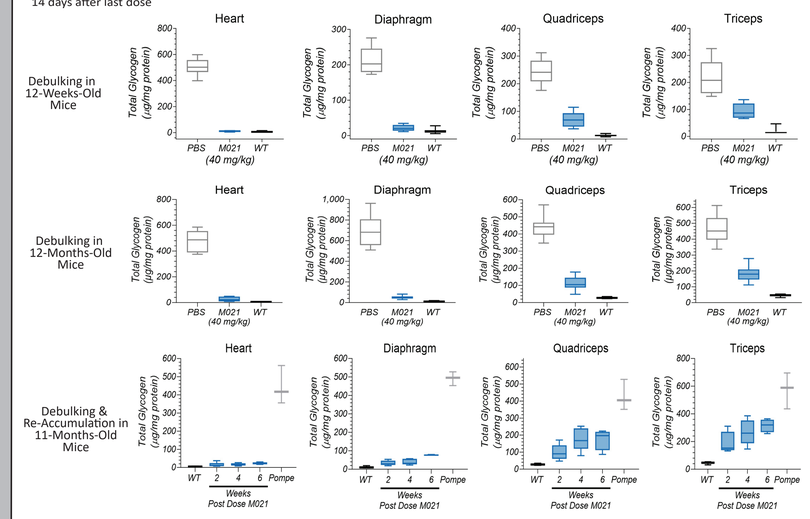


- Grip strength phenotype was significantly improved by M021 ERT by 2 months and indistinguishable from wild-type mice by 3 months.
- SOC was not able to improve grip strength under identical experimental conditions.

M021 May Offer Altenative Dosing Strategies for Rapid Debulking and Less Frequent Maintenance Dosing

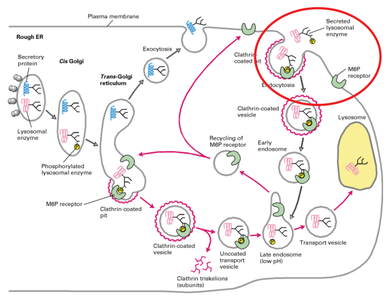
Animals
Strain: *Gaa*KO Pompe Mice
Gender: Mixed
N: 5- 11 per group
Age matched Wild-type
Treatment
M021- 40 mg/kg
6 weekly doses
EOL
14 days after last dose





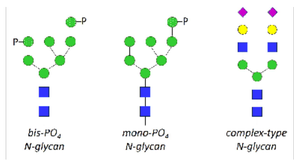
- Cardiac and diaphragm glycogen levles were normalized to Wild-type levels after M021 dosing, and very little glycogen had re-accumulated when measured 6 weeks after dosing.
- Glycogen in quadriceps and triceps were reduced 75% and 62%, respectively after M021 dosing compared to untreated Pompe mice.
- After glycogen debulking with M021, the rate of glycogen re-accumulation in skeletal muscle is slow (re-accumulated to ~50% of untreated Pompe mice 4-6 weeks after last dose).

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



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

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

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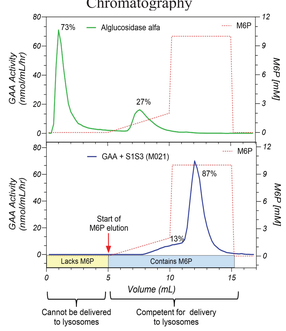
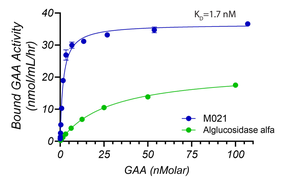
M021: rhGAA^{M6P} CI-MPR Interaction and Affinity is Dramatically Improved with Optimal Glycan Structure

CI-MPR Affinity Chromatography

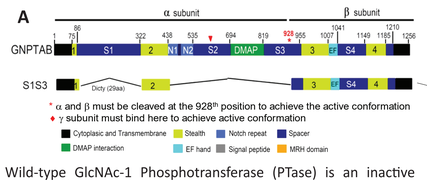


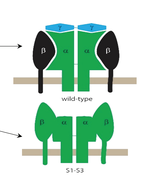
CI-MPR Binding Kinetics



- Only a portion (~ 27%) of alglucosidase alfa contains M6P and thus, only this minor fraction is able to bind to CI-MPR to enable cellular uptake. The poor affinity of Alglucosidase alfa (>100 nM) for the CI-MPR dramatically limits cellular uptake following therapy.
- In contrast, >99% of M021 contains M6P and has a strong affinity (1.7 nM) for the CI-MPR which enables efficient cellular uptake at relevant tissue levels following therapy.

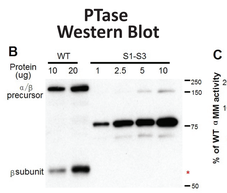
S1S3 Variant Has Key Attributes That Enable Its Use for Development of Best-In-Class Recombinant Enzymes and Gene Therapies



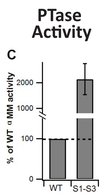


- Wild-type GlcNAc-1 Phosphotransferase (PTase) is an inactive zymogen that requires proteolytic cleavage at a precise location (at residue 928) to achieve its active conformation.
- No known way to increase proteolytic cleavage and enzyme activation within cells for increasing enzyme activity and improving phosphorylation.
- S1S3 PTase is a truncated, pre-activated variant with significant advantages:
  - Has proper conformation without need for gamma 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.

PTase Western Blot



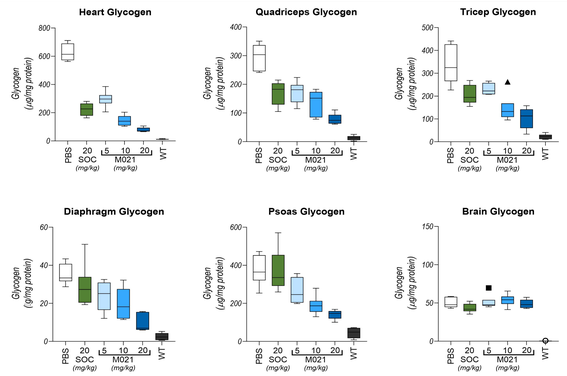
PTase Activity



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.

M021 is Significantly Better than SOC for Reducing Accumulated Glycogen in Short-term Efficacy Studies

Animals
Strain: *Gaa*KO Pompe Mouse Model
Gender: Mixed
Age: 12-14 weeks
N: 6 per group, 30 total
Age matched Wild-type
Treatment
PBS
SOC: Alglucosidase alfa 20 mg/kg
M021: 5, 10 and 20 mg/kg
2 Doses
EOL
14 days after last dose



- In the cardiac and skeletal muscle, M021 (20 mg/kg) is significantly better at reducing glycogen after 2 doses. Unfortunately the brain glycogen was not reduced.

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, resulting in high affinity binding to the CI-MPR receptor.
- Pre-clinical studies of M021 in the Pompe mouse model demonstrated robust efficacy as measured by in-life grip strength and glycogen reduction.
- M021 effectively reduces glycogen, even in aged Pompe mice with advanced disease phenotype.
- These promising results suggest that M021 warrants further development as a potential next-generation treatment for Pompe disease.