Poster # P138

M021: Pompe Enzyme Replacement Therapy with Highly Phosphorylated Oligosaccharides by the Co-Expression of Acid Alpha-Glucosidase with S1S3 GlcNAc-1-Phosphotransferase

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Abstract

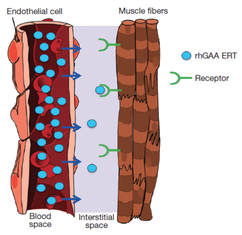
Pompe disease is a rare inherited metabolic disorder of defective lysosomal glycogen catabolism due to a deficiency in acid alpha-glucosidase (GAA). Alglucosidase alfa enzyme replacement therapy (ERT) using recombinant human GAA (rhGAA ERT) has provided clinical benefits, but these benefits have been limited primarily due to poor drug targeting of ERT to skeletal muscles. Recent therapeutic approaches (Cipaglucosidase alfa and Avalglucosidase alpha) have improved cation-independent mannose-6 phosphate receptor (CI-MPR) targeting by increasing the number of phosphorylated oligosaccharides present on rhGAA. Cipaglucosidase alfa is produced using a unique CHO cell line that produces rhGAA with increased phosphorylated oligosaccharides coupled with a highly engineered manufacturing process that yields an ERT with at least one bis-phosphorylated oligosaccharide per molecule of protein. Avalglucosidase alpha is a chemically engineered rhGAA produced by attaching synthetic bis-phosphorylated oligosaccharides to rhGAA utilizing a chemical conjugation process exploiting oxidized sialic acid residues.

M021 is a novel rhGAA co-expressed with S1S3-phosphotransferease in CHO cells being developed by M6P Therapeutics. By utilizing the S1S3-phosphotransferase, GAA phosphorylation is greatly enhanced in the production cell line. Since phosphorylation is no longer a challenge, this more allows more focus on optimizing the entire oligosaccharide profile of M021. Currently we have developed a cell line and process that yields a rhGAA that has >90% of its oligosaccharides phosphorylated with >70% being bis-phosphorylated. This is of importance since bis-phosphorylated have the greatest affinity for the CI-MPR. In addition to the high level of bis-phosphorylated oligosaccharides, M021 have very low levels of neutral oligosaccharides (<5%). This unique oligosaccharide profile should maximize CI-MPR targeting while minimizing unproductive mannose receptor and asialoglycoprotein receptor clearance in the liver.

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 ultra-rare autosomal recessive disorder
- Pompe disease is caused by low levels or absence of acid α-glucosidase (GAA) activity, 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

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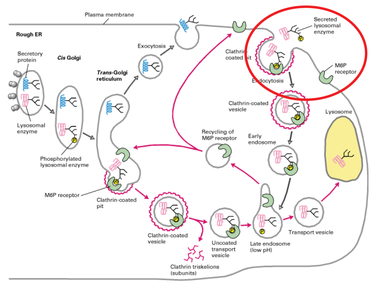
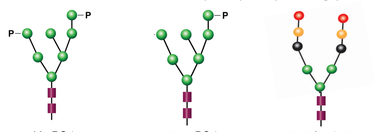
CI-MPR is the Main Receptor Responsible for Targeting Lysosomal Proteins to Lysosomes



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.
Bis-phosphorylated glycans have ~3,000x greater affinity for the CI-MPR than Mono-phosphorylated glycans



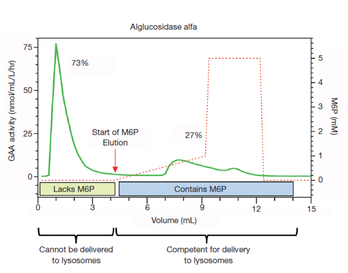
bis-PO4 N-glycan *mono-PO4 N-glycan* *complex-type N-glycan*

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

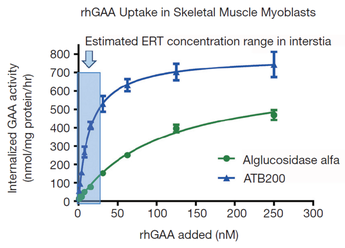
Do et al. Ann Transl Med 2019: 7(13):291

Alglucosidase Alfa is a Poor Ligand for the CI-MPR

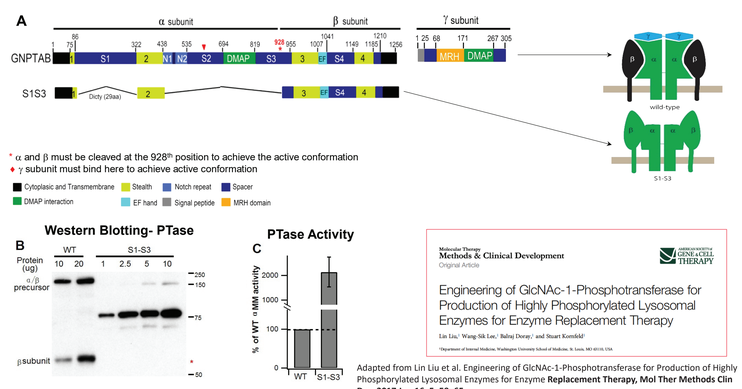
Only about 1 in 4 GAA molecules can bind the CI-MPR



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

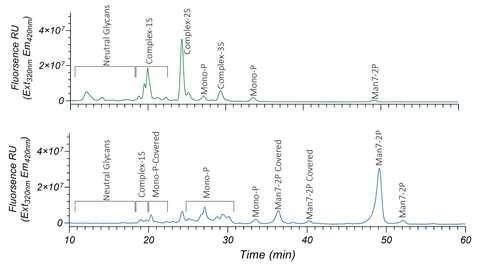


Molecular Therapy Methods & Clinical Development
Original Article
Engineering of GlcNAc-1-Phosphotransferase for Production of Highly Phosphorylated Lysosomal Enzymes for Enzyme Replacement Therapy
Lin Liu,[1] Wang-Sik Lee,[1] Balraj Doray,[1] and Stuart Kornfeld[1]
[1]Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA

Adapted from 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: Pompe Enzyme Replacement Therapy with Optimized Oligosaccharides

2AB Oligosaccharide Mapping



Glycan Composition Summary

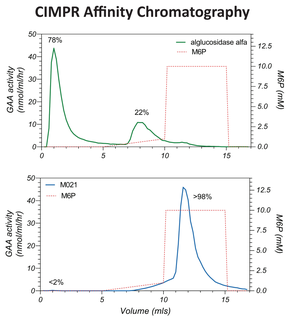
N-Glycan Types	SOC		M021	
	(% of Total)	(mol/mol GAA)	(% of Total)	(mol/mol GAA)
Neutral	12%	0.8	<1%	<0.1
Complex Charges	83%	5.4	8%	0.5
Mono-PO4	3%	0.2	31%	2.0
Mono-PO4 Covered	*ND*	-	3%	0.2
Bis-PO4	2%	0.5	46%	3.0
Bis-PO4 Covered	*ND*	-	12%	0.8

ND: Not Detected

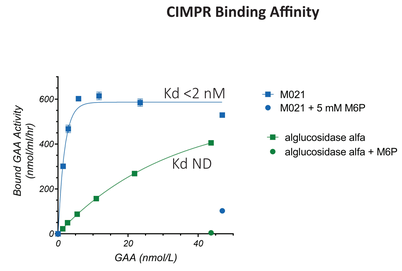
- Historically, endogenous and rhGAA are poorly phosphorylated
- Regardless of the expression system, rhGAA is highly phosphorylated when co-expressed with S1S3 GlcNAc-Phosphotransferase
- >85% of the N-linked glycans found on M021 are phosphorylated. With >50% being bis-phosphorylated resulting in very 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.

M021 has High Affinity for the CIMPR

CIMPR Affinity Chromatography



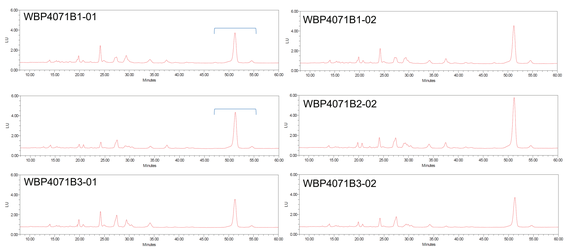
CIMPR Binding Affinity



- Greater that 98% of M021 can interact with the CIMPR
- The amount of M6P had to be increased to efficiently elute M021 in the CIMPR affinity chromatography assay.
- A decrease in pH was more effective in eluting M021 than using the competitive inhibitor. Reflective of the normal intracellular process

M021: Single Cell Clones are even more highly phosphorylated than stable pool material produced so far.

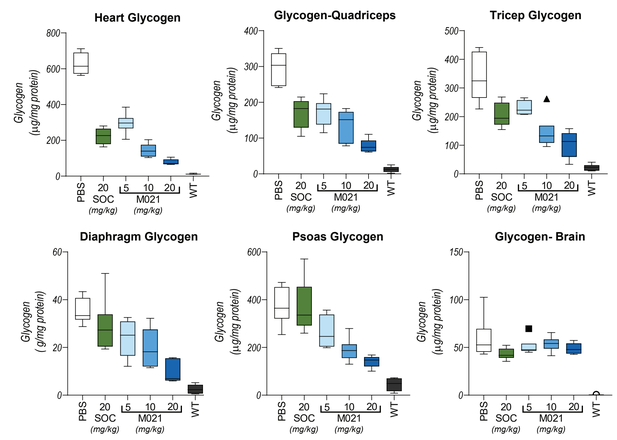
Stable Pools Used For Cloning (2AB Mapping of Partially Purified GAA)



ID	Others	Neutral: Man5	Neutral: G2	Neutral: G2F	Complex Sialylated: G2S1	Complex Sialylated: G2F1S1	Complex Sialylated: G2F2S2	GlcNAc Covered Mono-PO4: 3M000-1P	GlcNAc Covered Mono-PO4: 3T000-1P	GlcNAc Covered Mono-PO4: 4T110-1P	GlcNAc Covered Mono-PO4: 3T110-1P	Mono-PO4: Man5-1P	Mono-PO4: Man6-P	Mono-PO4: Man7-P	GlcNAc Covered Bis-PO4: 3T000-2P	GlcNAc Covered Bis-PO4: 3M000-2P	GlcNAc Covered Bis-PO4: 4T000-2P	Bis-PO4: Man6-2P	Bis-PO4: Man7-2P	Bis-PO4: Man8-2P	Sum Bis-PO4	Total Bis-PO4 + Covered-PO4
Stable Pool Initial	5.0	2.4	1.3	0.5	0.7	2.3	4.2	2.5	0.7	3.3	2.9	6.7	5.4	1.6	4.7	1.3	0.5	2.1	49.3	2.7	54.1	60.6
4071B-01-02	3.0	0.7	.	0.4	1.0	0.6	0.9	3.4	1.2	0.8	0.7	3.1	9.1	2.0	17.0	2.8	0.8	4.3	45.1	2.4	51.8	72.4
4071B-01-05	3.7	0.7	0.6	0.5	0.8	0.5	0.8	2.8	1.4	0.4	0.6	2.8	12.3	2.4	22.0	2.2	1.0	4.0	38.9	1.5	44.4	69.6
4071B-01-16	3.5	0.6	0.5	0.5	1.0	0.7	1.0	3.7	1.2	0.7	0.8	3.4	8.8	2.2	15.8	2.9	0.7	4.3	45.5	2.2	52	71.4
4071B-01-20	3.2	0.6	0.4	0.4	1.0	0.7	1.3	3.2	1.0	0.8	1.0	3.3	8.1	2.2	15.8	2.8	0.9	4.8	46.2	2.3	53.3	72.8
4071B-02-01	3.2	0.9	0.7	0.4	0.6	0.4	0.6	2.4	1.4	0.8	0.6	3.1	9.6	2.1	15.7	2.0	0.8	5.0	47.9	1.8	54.7	73.2
4071B-02-08	3.8	0.5	0.4	0.4	0.6	0.4	0.5	2.3	1.3	0.6	0.6	2.6	11.1	2.3	19.4	2.0	0.9	4.7	44.1	1.7	50.5	72.8
4071B-02-14	3.7	0.6	0.4	0.3	0.6	0.4	0.7	2.2	1.1	0.7	0.7	2.8	10.5	2.4	18.8	1.9	0.8	5.1	44.9	1.7	51.7	73.2

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

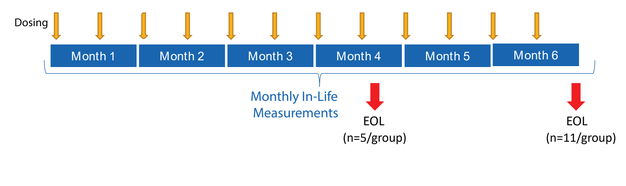
Animals
Strain: GAAKO Pompe Mouse Model
Gender: Mixed
Age: 12-14 weeks
N: 6 per group, 30 total
Age matched Wild-type
Treatment
PBS
SOC: Alglucosidase alfa
20 mg/kg
M021:
5, 10 and 20 mg/kg
2 Doses
EOL
14 days after last dose



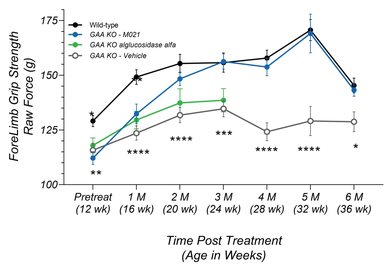
In the Cardiac and Skeletal Muscle, M021 is significantly better at reducing glycogen. Unfortunately the brain glycogen was not reduced.

M021: Long Term Efficacy Evaluated

Animals
Strain: *Gaa* KO Pompe Mouse Model
Gender: Mixed
Age: 12-14 weeks
N: 6 per group, 36 total
Treatment
Age matched Wild-type
PBS
SOC: Alglucosidase alfa
20 mg/kg
M021
20 mg/kg
7 Doses
EOL : 70 days after last dose
In-Life
Grip Strength
EOL
14 days after last dose
Activity
Glycogen
PAS Staining



M021 Therapy Starts Restoring Grip Strength by 2 Months, with Complete Restoration by 3 Months



Amicus AT-GAA Published Data

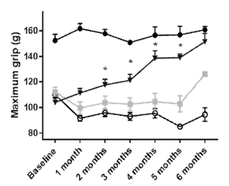
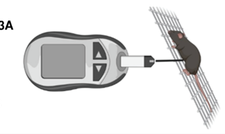


FIG. 33A



- Animals receiving M021 started to normalize grip strength by 2 months
- Animals receiving M021 had grip strength comparable to wild-type animals by 3 months

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

- WuXi Apptec and WuXi Biologics

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