Poster # 142

M021: rhGAA with optimal glycosylation profile containing very high levels of bis-phosphorylated N-glycans clears accumulated glycogen and rapidly normalizes muscle strength in treated Pompe mice

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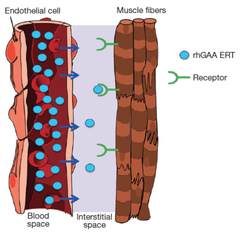
Abstract

Pompe disease is a rare inherited metabolic disorder of defective lysosomal glycogen catabolism caused by deficiency in acid alpha-glucosidase (GAA). Alglucosidase alfa enzyme replacement therapy (ERT) using recombinant human GAA (rhGAA) has provided irrefutable clinical benefits, but the therapy is sub-optimal due to poor cellular uptake of ERT into skeletal muscles. It is estimated that only ~1% of rhGAA ERT reaches the interstitial space surrounding muscles to yield very low resultant enzyme concentrations post-dosing which necessitates an efficient mechanism for cellular uptake. Specialized carbohydrate structure called mannose 6-phosphate (M6P), particularly bis-phosphorylated N-glycan structures are needed for binding cation-independent M6P receptor (CI-MPR) at such low enzyme concentrations to enable cellular uptake of exogenous ERT into muscle cells. rhGAA is inherently poorly phosphorylated and there has not been a reliable way to modulate N-glycan processing within cells to produce highly phosphorylated rhGAA. We have developed a novel process to co-express rhGAA with truncated GlcNAc-1-phosphotransferase (S1S3-PTase) for producing highly phosphorylated rhGAA (designated as M021) where >90% of all glycans are naturally phosphorylated, of which, >67% are bis-phosphorylated N-glycans which have the highest binding affinity for CI-MPR. M021 also contains very low levels (1%) of neutral glycans (non-phosphorylated high mannose and de-sialylated complex structures) for reduced non-productive clearance of ERT in liver and other non-target tissues. M021 was shown to be substantially better targeted to muscles than alglucosidase alfa for normalizing tissue glycogen. Further, M021 was shown to quickly normalize muscle grip strength of Pompe mice to that of WT mice by 2-3 months and maintained over 6 months while standard of care ERT could not under identical experimental conditions. This rapid improvement in muscle function is unprecedented and suggests that the unique glycosylation profile of M021 may enable optimal drug targeting for developing a much more potent ERT for Pompe disease.

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.

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

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

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

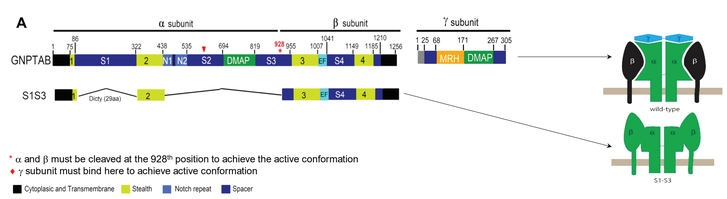
Only about 1 in 4 GAA molecules can bind the CI-MPR

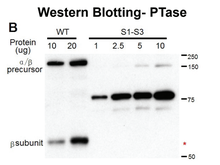
Do et al. Ann Transl Med 2019: 7(13):291

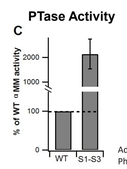
Affinity for the receptor is to poor for efficient binding at relevant tissue levels

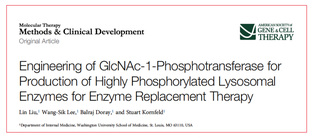
Do et al. Ann Transl Med 2019: 7(13):291

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



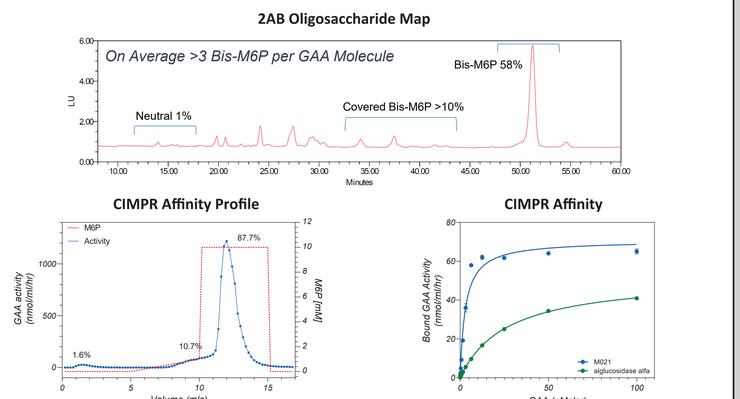






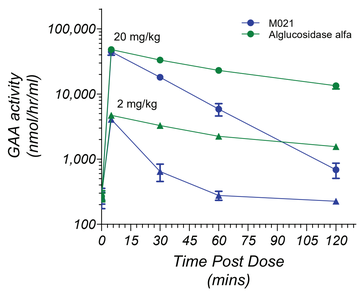
Adapted from Lin Liu et al. Engineering of GlcNAc-1-Phosphotransferase for Production of Highly Phosphorylated Lysosomal Enzymes for Enzyme Replacement Therapy, **Mol Ther Methods Clin Dev. 2017 Jun 16; 5: 59–65.**

M021: Pompe Enzyme Replacement Therapy with Optimized Oligosaccharides



- Regardless of the expression system, rhGAA is highly phosphorylated when co-expressed with S1S3 PTase
- >95% of the N-linked glycans found on M021 are phosphorylated, with >67% being bis-phosphorylated Resulting in very high affinity (<5 nM KD) for the CI-MPR ensuring efficient cellular uptake

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



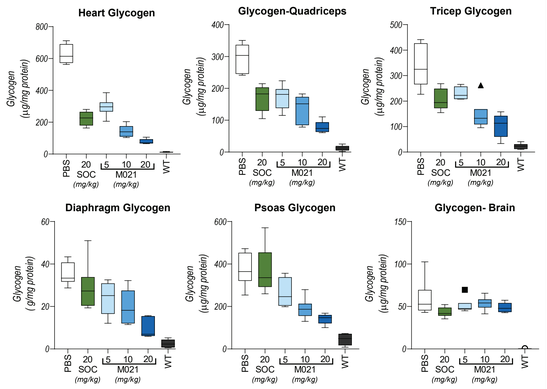
20 mg/kg	Alglucosidase alfa	M021
Half-life (min)	58	25
AUC (nmol/hr/ml)	3,079,725	1,451,363

2 mg/kg	Alglucosidase alfa	M021
Half-life (min)	55	18
AUC (nmol/hr/ml)	309,636	99,069

- 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

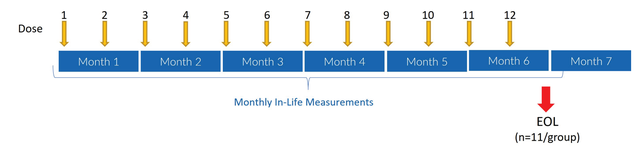
Animals
Strain: *Gaa*KO Pompe Mouse Model
Gender: Mixed
Age: 12-14 weeks
N: 6 per group, 30 total
Age matched Wild-type
Treatment
PBS
SOC: Alglucosidase alfa 20 mg/kg
M021:
5, 10 and 20 mg/kg
2 Doses
EOL
14 days after last dose



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

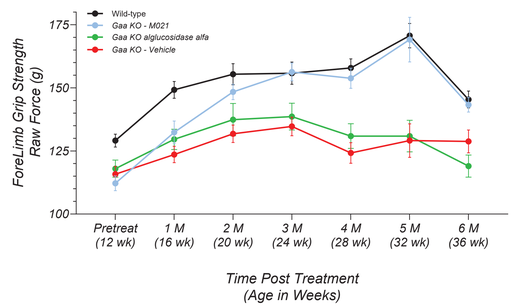
M021: Long Term Efficacy Evaluated

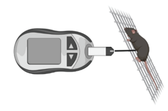
Animals
Strain: *Gaa*KO Pompe Mouse Model
Gender: Female
Age: 12-14 weeks
N: ~11 per group
Treatment
Age matched Wild-type
PBS
Alglucosidase alfa @ 20 mg/kg
M021 @ 20 mg/kg
13 Doses
In-Life
Grip Strength
EOL
14 days after last dose
Activity
Glycogen - In Process



Lorem ipsum

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

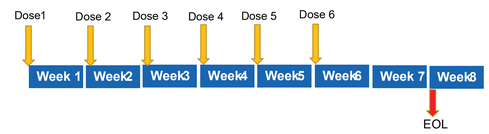


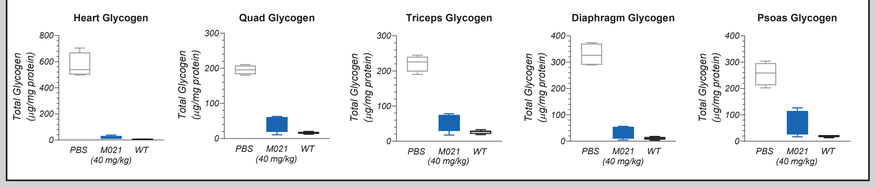


- Grip strength phenotype was significantly improved by M021 ERT by 2 months and indistinguishable from wild-type mice by 3 months.

M021 Normalized Glycogen Levels to Near WT Levels in Skeletal Muscles in 12M Old Pompe Mice after 6 doses

Animals
Strain: *Gaa*KO Pompe Mouse Model
Gender: Female
Age: 12 months
N: 4 per group
Age matched Wild-type
Treatment
PBS
M021 @ 20 mg/kg
6 Weekly doses
EOL
14 days after last dose





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
- Pre-clincial studies of M021 in the Pompe mouse model demonstrated robust efficacy as measured by in-life grip strength and glycogen reduction
- Significant glycogen reduction was observed in older Pompe mice
- These promising results suggest that M021 warrants further development as a potential next-generation treatment for Pompe disease

Acknowledgments

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