Highly phosphorylated β-glucocerebrosidase (M011) has much broader tissue targeting and superior substrate reduction with potential for alternative dosing strategies for the treatment of Type I Gaucher disease

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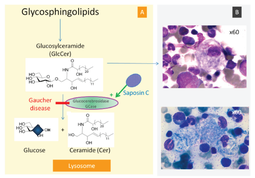
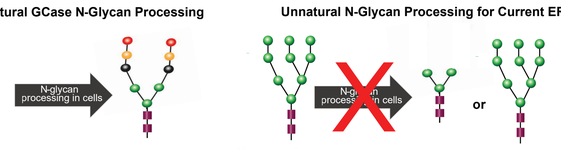
Gaucher Disease

- Gaucher disease is one of the most common lysosomal storage diseases (LSDs) and results from the defective of GBA (GCase) enzyme. GCase enzyme breaks down glycosylceramide (GlcCer) to glucose and ceramide in the lysosome.
- Three major clinical types of Gaucher disease are identified
  - Gaucher type I: most common. With clinical evidence of bone disease, lung disease, etc, but absence of primary central nervous system disease.
  - Gaucher types II & III are characterized by the presence of primary neurologic disease. Disease with onset before age 2 and a rapidly progressive course is classified as type II. otherwise, it is type III.



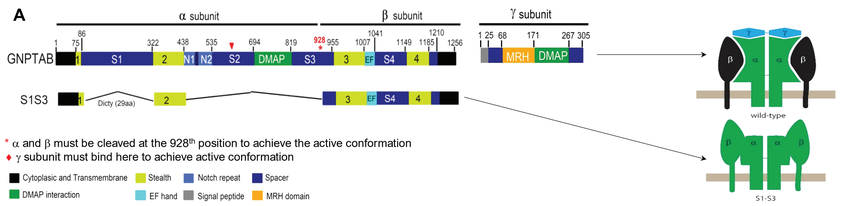
Int J Mol Sci. 2017 Feb 17;18(2)

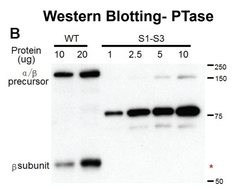
GCase is Naturally Produced with Complex-Type N-Glycans that do not Enable Cellular Uptake; Current ERTs Require Unnatural Glycan Processing to Enable Cellular Uptake into Macrophages

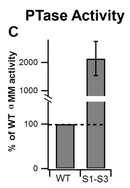


- Targeting the mannose receptors has been effective for Type I Gaucher
- Uptake limited to cells with mannose receptors.

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







Molecular Therapy Methods & Clinical Development
Original Article

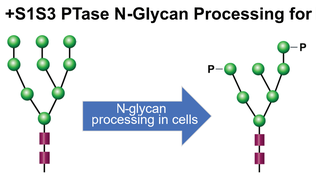
Engineering of GlcNAc-1-Phosphotransferase for Production of Highly Phosphorylated Lysosomal Enzymes for Enzyme Replacement Therapy

Lin Liu,[1] Wang-Sik Lee,[1] Baltaj Doray,[1] and Stuart Kornfeld[1]

[1]Department of Internal Medicine, Washington University School of Medicine, St. Louis, MO 63110, USA

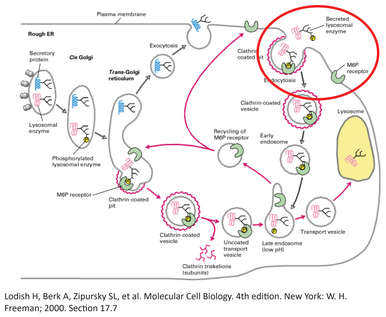
Adapted from Lin Liu et al. Engineering of GlcNAc-1-Phosphotransferase for Production of Highly Phosphorylated Lysosomal Enzymes for Enzyme **Replacement Therapy, Mol Ther Methods Clin Dev. 2017 Jun 16; 5: 59–65.**

Co-expression of S1S3 PTase Naturally Produces rhGCase with High Levels of M6P



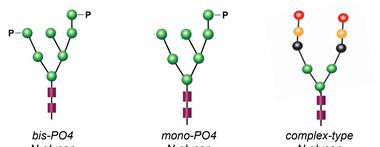
- M6P is the natural biological motif for binding M6P receptors to facilitate cellular uptake and lysosomal delivery for most soluble lysosomal enzymes

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

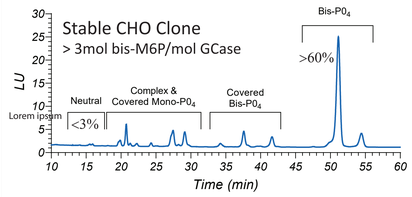
Not all phosphorylated glycans are equal.
Bis-phosphorylated glycans have ~3,000x greater affinity for the CI-MPR than Mono-phosphorylated glycans



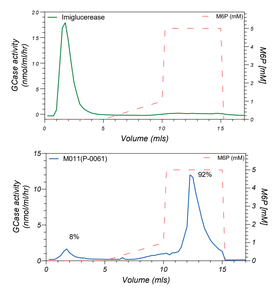
Ligand	Binding affinity (Apparent K_D; nM)*
bis-phosphorylated N-glycan	2
mono-phosphorylated N-glycan	>6000
complex type N-glycan	No binding

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

M011: Novel GCase ERT with Phosphorylated N-glycans Enables Cellular Uptake into Nearly All Cell Types via CI-MPR



Enzyme	Activity (U)	CI-MPR Kd)
Cerezyme	11.9	>200 nmolar
M011	16.6	<5 nmolar



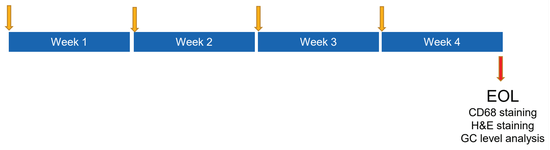
- Oligosaccharides are fundamentally distinct than other GCase ERTs and more representative of other soluble lysosomal enzymes
- >90% of the N-linked glycans found on M011 are phosphorylated. With >60% being bis-phosphorylated resulting in very high affinity (<5 nM KD) for the CI-MPR that ensure efficient cellular uptake
- M011 is significantly more stable and has higher specific activity than WT GCase

M011 is More Potent than Imiglucerase for Reducing CD68+ Storage Cells After 4 Doses in Gaucher Mouse Model

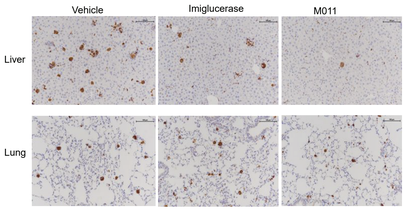
Animals
Strain: *D409V/null* Gaucher Mouse Model
Gender: Mixed
Age: 18 weeks
N: 3-8 per group

Treatment
PBS
SOC: Imiglucerase @ 1.5 mg/kg
M011 @ 1.5 mg/kg
4 Doses weekly (total of 4 doses)
EOL : 1 weeks after last dose

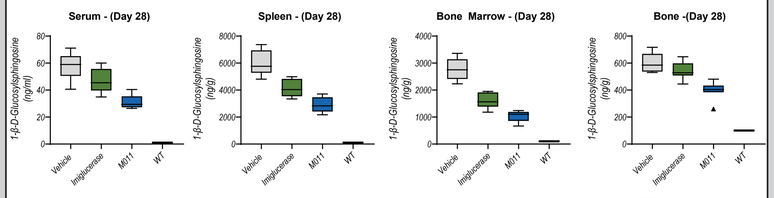
EOL
CD68 staining
H&E staining
GC level analysis



M011 is more effective for reducing macrophage storage cells as shown by anti-CD68+ IHC



M011, a well phosphorylated and differentiated glucocerebrosidase, has superior substrate clearance vs. current SOC after 28 days of treatment



- M011 ERT reduced tissue macrophages infiltration better than imiglucerase in the GBA^{D409V} mouse
- M011 ERT reduced the number and size of gaucher storage cells better than imiglucerase in the GBA^{D409V} mouse
- M011 ERT improved substrate clearance in key tissues compared to imiglucerase in the GBA^{D409V} mouse

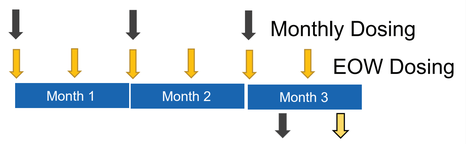
M011 Dosed Monthly May Achieve Same Level of Efficacy as the Standard of Care Dosed Bi-Weekly

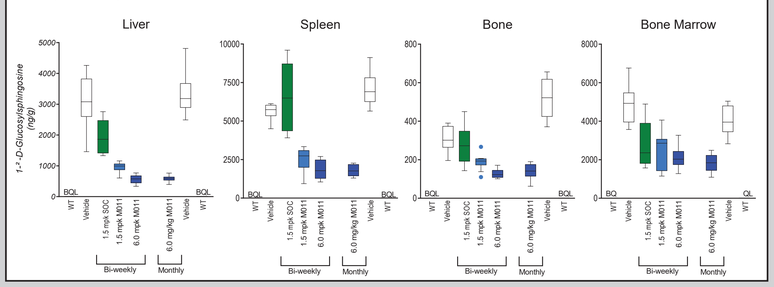
Animals:
Strain: *D409V/null* Gaucher Mouse Model
Gender: Mixed
Age: 12-14 weeks
N: 10 per group
Age-matched WT and untreated controls

Treatment:
Schedule 1- dosed EOW, IV slow push
Imiglucerase @ 1.5 mg/kg
M011 GCase @ 1.5 mg/kg
M011 GCase @ 6 mg/kg
Age-matched WT and untreated controls
Schedule 2- dosed monthly, IV slow push
M011 GCase @ 6 mg/kg

EOL: 1 week after last dose

Analysis: Activity and Substrate

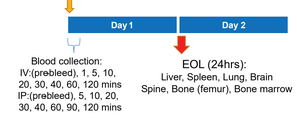




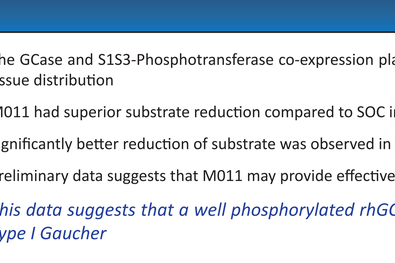
IP Dosing for M011 Allows for Greater Tissue Uptake

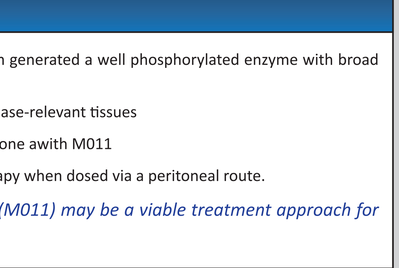
Animals:
Strain: *D409V/null* Gaucher Mouse Model
Gender: Mixed
Age: 12-16weeks
N: 6 per group

Treatment:
Single IV dose:
Vehicle
M011 @ 6 mg/kg
Imiglucerase @ 1.5 mg/kg
Single IP dosing:
M011 @ 6 mg/kg + 30 μM IFG
M011 @ 9 mg/kg + 30 μM IFG
In-Life: Serum Activity
EOL: 24 hr after dose
Analysis: Tissue and Serum Activity



Treatment	Route	Dose (mg/kg)	C_{max} (nmol/ml/hr)	C_{max} (nM)	AUC (nmol/ml/hr)	$T_{1/2}$ (min)
M011 + IFG	IP	6 + 30	14,218	757	903,478	14
M011 + IFG	IP	9 + 30	18,846	1,003	1,501,264	13
M011	IV	6	40,941	2,180	780,947	6
M011	IV	1.5	15,552	828	146,908	8
Imigluceraase	IV	1.5	2,136	113	13,456	5



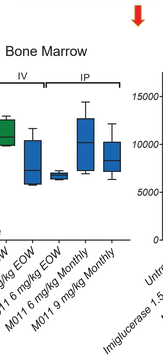


- M011 has optimal glycan profile that yields a much more favorable pharmacokinetics and exposure than imiglucerase
- M011 dosed at 6 mg/kg have similar Exposure (AUC) and Half-life (T) regardless of dosing route
- M011 IP dosing due to it slower appearance in the blood allow for greater time for tissue uptake
- M011 dosed at 6 mg/kg IP has greater tissue uptake than the same dosed administered by IV in most tissue

Lyso-GL1 Levels Are Reduced Effectively at 6 mg/kg and EOW IP Dosing is as Effective as EOW IV Dosing

Animals:
Strain: *D409V/null* Gaucher Mouse Model
Gender: Mixed
Age: 12-16weeks
N: 5 per group
Age-matched WT and untreated controls

Treatment:
IV dosing (bi-weekly):
Vehicle
M011 @ 6 mg/kg
Imiglucerase @ 1.5 mg/kg
IP dosing (bi-weekly)
Vehicle
M011 @ 6 mg/kg + 30 μM IFG
IP dosing (monthly)
M011 @ 6 mg/kg + 30 μM IFG
M011 @ 9 mg/kg + 30 μM IFG
EOL: 2 weeks after last dose
Analysis: Substrate



- M011 ERT at 6 and 9 mg/kg effectively reduced Lyso-GL1
- M011 ERT dosed at 6 mg/kg was most effective at reducing Lyso-GL1
- M011 ERT dosed at 6 mg/kg IP, appears to lower Lyso-GL1 more robustly in Bone Marrow

Summary

- The GCase and S1S3-Phosphotransferase co-expression platform generated a well phosphorylated enzyme with broad tissue distribution
- M011 had superior substrate reduction compared to SOC in disease-relevant tissues
- Significantly better reduction of substrate was observed in the Bone awith M011
- Preliminary data suggests that M011 may provide effective therapy when dosed via a peritoneal route.

This data suggests that a well phosphorylated rhGCase (M011) may be a viable treatment approach for Type I Gaucher