Highly phosphorylated β-glucocerebrosidase (M011) That Targets Central Nervous System Neurons as a Potential Treatment for Neuronopathic Gaucher's Disease Type 2 and 3

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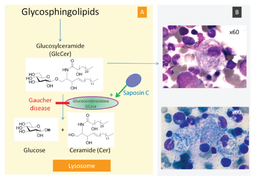
Gaucher Disease

- Gaucher disease is one of the most common lysosomal storage diseases (LSDs) and results from a defective GBA (GCase) enzyme. GCase breaks down glycosylceramide (GlcCer) to glucose and ceramide in the lysosome.
- Three major clinical types of Gaucher disease are identified
  - Gaucher type 1 is the most prevalent. With clinical evidence of bone disease, lung disease, etc, but absence of primary central nervous system disease.
  - Gaucher types 2 & 3 are characterized by the presence of primary neurologic disease. Disease with onset before age 2 and a rapidly progressive course is classified as type 2, otherwise, it is type 3. No ERT or GTx therapies are approved for Gaucher types 2 and 3.



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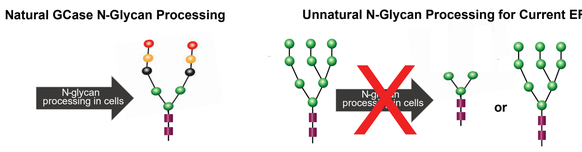
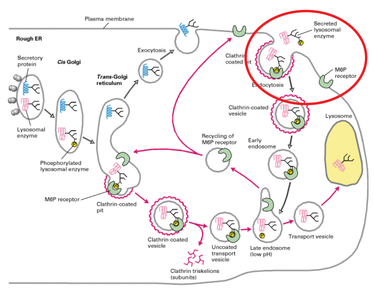
GCase is Naturally Produced with Complex-Type N-Glycans that do not Enable Cellular Uptake; Current ERTs Require Unnatural Glycan Processing to Enable Cellular Uptake into Macrophages



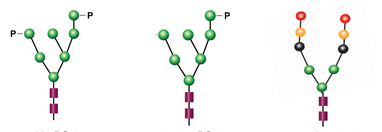
- Targeting the mannose receptors has been effective for Type I Gaucher
- Uptake limited to cells with mannose receptors.

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

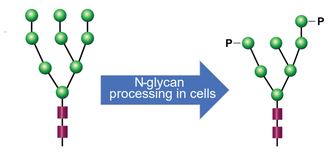


Ligand	Binding affinity (Apparent K_D; nM)*
bis-phosphorylated N-glycan	2
mono-phosphorylated N-glycan	>6000
complex type N-glycan	No binding

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

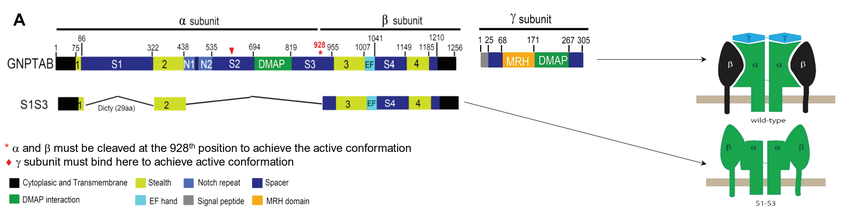
Co-expression of S1S3 PTase Naturally Produces rhGCase with High Levels of M6P

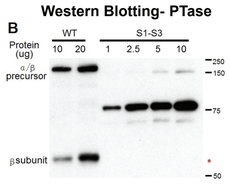
+S1S3 PTase N-Glycan Processing for M011

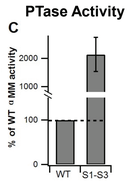


- M6P is the natural biological motif for binding M6P receptors to facilitate cellular uptake and lysosomal delivery for most soluble lysosomal enzymes

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

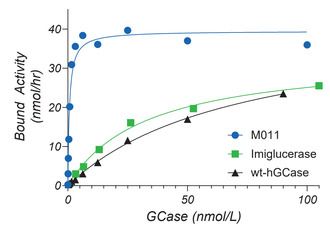
Lin Liu,[1] Wang-Sik Lee,[1] Balraj Doray,[1] and Stuart Kornfeld[1]

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

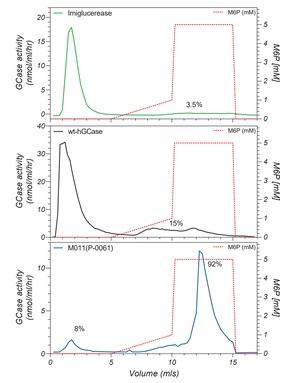
Adapted from Lin Liu et al. Engineering of GlcNAc-1-Phosphotransferase for Production of Highly Phosphorylated Lysosomal Enzymes for Enzyme **Replacement Therapy, Mol Ther Methods Clin Dev. 2017 Jun 16; 5: 59–65.**

M011: Novel GCase ERT with Phosphorylated N-glycans for Cellular Uptake via CI-MPR

CI-MPR Affinity Determination



CI-MPR Affinity Chromatography

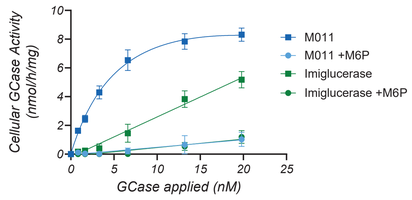


Enzyme	CIMPR Binding (%)	CIMPR Kd (nM)
Imiglucerase	~3.5	>200
Wt-hGCase	~15	>300
M011	>90	<5

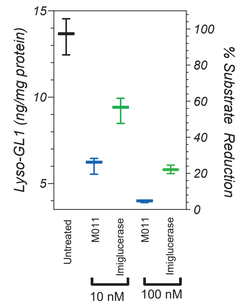
- Oligosaccharides found on M011 are fundamentally distinct than other GCase ERTs and more representative of other soluble lysosomal enzymes
- >90% of the N-linked glycans found on M011 are phosphorylated. With >60% being bis-phosphorylated resulting in very high affinity (<5 nM KD) for the CI-MPR that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

M011 has Better Neuronal Cell Uptake and More Effective Lyso-Gl1 Reduction then Imiglucerase in Gaucher Fibroblast Cells

GCase Cell Uptake in HTB-186 Neuronal Cells



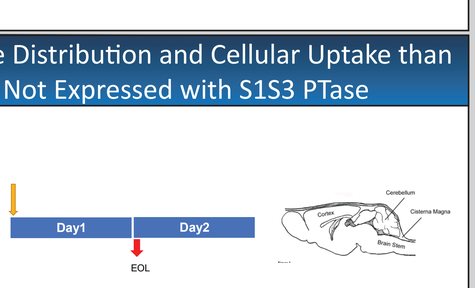
Lyso-GL1 Levels in Type 2 Gaucher Fibroblasts



- M011 Kuptake is <3 nMolar while Imiglucerase >10 nM
- M011 was 10x more efficient at reducing Lyso-GL1 levels in neuronal cell line
- M011 should be effective in reducing neuronal substrate if 10nM interstitial concentrations can be achieved in the CNS

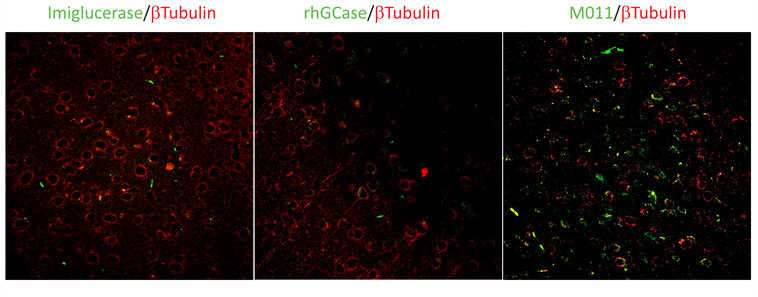
M011 Has Greater Tissue Distribution and Cellular Uptake than Imiglucerase or rhGCase Not Expressed with S1S3 PTase

Animals:
Strain: *D409V/null* Gaucher Mouse Model
Gender: Female
Age: 10-12 weeks
N: 3-4 per group
Age-matched untreated controls
Treatment:
ICM dosing:
Vehicle
M011 @ 90 μg/mouse
rhGCase @ 90 μg/mouse
Imiglucerase @ 90 μg/mouse
EOL: 24 hrs post dose
Analysis: GCase IHC



ICM delivered M011 colocalizes with visual cortex neurons

Imiglucerase/βTubulin | rhGCase/βTubulin | M011/βTubulin

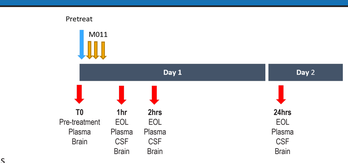


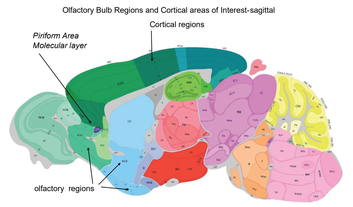
M011 is Significantly Increased in Cortical Neurons 2 hours Post-Intranasal Delivery in D409V mice

Animals:
Strain: *Gba*^{D409V}/*Gba*^{D409V} Gaucher Mouse Model
Gender: Female
Age: 7 months
N: 5 per group
Age-matched WT and untreated controls
Treatment:
Procedure
Pre-treatment with 5 μL Hyaluronidase per nostril 30 min prior to test article
Test Article administration 3 μL per nostril every 10 mins for total of 3 administrations
Dosing Solutions
Hyluronidase @10 mg/ml
PBS Vehicle
M011 @ 18 mg/ml +30 μM IFG

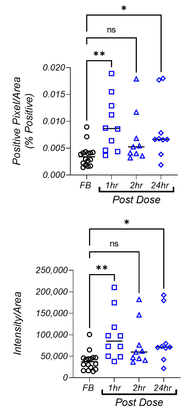
EOL: 2 hours after last dose

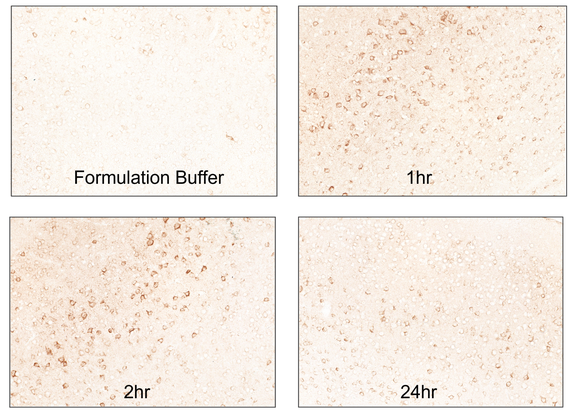
Analysis: GCase IHC





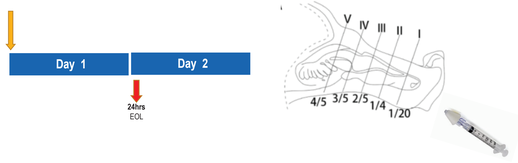
IHC Staining of Cortical Neurons 1, 2 and 24 Hours Post IN Delivery

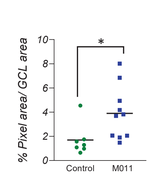


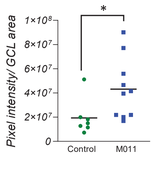


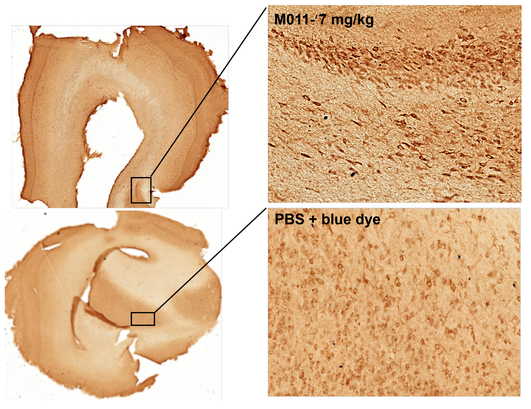
M011 is Significantly Increased in Yucatan Pig Olfactory Bulb Glomerular Cell Layers 24 Hours Post IN Delivery

Animals:
Pig: Yucatan Herd
Gender: Female
Age: 5 months
Mass: 14-21 kg
N: 4 treated, 2 controls
Treatment:
Procedure
Pre-treatment: Lidocaine 0.5 mL/nostril
Single IN Dose, 5mL/nostril
MAD Nasal IN Musocal Atomization Device
Treatment Groups
Control: Methylene Blue dye + PBS
M011 @ 7.0 mg/kg
EOL: 24 hr after dose
Analysis: IHC









- M011 delivered intranasal is present in olfactory bulb neurons
- M011 was present 24 hrs after intranasal delivery
- Represents a significant increase over endogenous GCase levels
- Intranasal may prove to be a viable dosing route for M011 delivery to the CNS

Summary

- M011's high level of phosphorylated glycans allow it to be taken up by neuronal cells via CI-MPR
- ICM dosing illustrates that M011 can be taken up by CNS neurons distant from delivery site
- Intranasal dosing shows promise in both mice and pigs as a therapeutic dosing route.
- Additional work is warranted in exploring possibility of intranasal delivery of M011 for Gaucher Type II and III ERT