Brain penetrant structurally targeted allosteric regulators for treating Parkinson disease and other α-synucleinopathies

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INTRODUCTION and PURPOSE

GBA1 gene encodes the lysosomal enzyme GCase involved in the metabolism of sphingolipids. Pathological mutations in this gene, which lead to reduced GCase activity, are the most common genetic risk factor for Parkinson's disease (PD) and promote α-synuclein accumulation in the brain, a hallmark of PD and other α-synucleinopathies. Thus, augmentation of GCase activity is considered a therapeutic strategy for advancing drug discovery in α-synucleinopathies including PD.

Pharmacological chaperones (PCs) are small molecules that stabilize the defective enzyme, avoiding its degradation and recovering its enzymatic activity. PCs might also bind the wild-type GCase protein and increase its activity. Allosteric non-inhibitory PCs offer advantages when compared with competitive PCs, normally sugar-like, by not competing with the natural substrate and potentially showing better drug-like properties, especially for CNS indications.

The company Gain Therapeutics has applied its innovative proprietary platform, Site-directed Enzyme Enhancement Therapy (SEE-Tx), to discover new pharmacological chaperones capable of restoring protein function. Gain's SEE-Tx technology identifies and exploits druggable allosteric sites different from the active-site

Our purpose is **to develop novel brain-penetrant allosteric GCase chaperones for the treatment of GBA-related diseases such as Parkinson Disease and other α-synucleinopathies**

DISCOVERY STRATEGY

Identification of a new allosteric binding site

- The published human GBA 3D structure obtained by X-ray crystallography and refined to 1.95 Å resolution was used (PDB ID: 2v3f).
- A previously undescribed druggable cavity was identified with a physics-based method consisting in molecular dynamic simulations of the protein in organic-aqueous solvent mixtures, called **MDmix**.
- The same method was used to identify key interaction sites (binding hot spots), which were used as pharmacophoric restraints to guide docking.

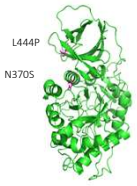

Figure 1. GCase monomer 3D structure.

Hit ID by Virtual Screening

- A virtual collection of >6 million commercially available compounds were evaluated computationally with the docking program **rDock** using the standard scoring function, pharmacophoric restraints and a high-throughput protocol.
- Best scoring compounds were visually inspected and a subset of them was selected based on the plausibility of the binding mode and chemical diversity considerations.
- Finally, 96 virtual hits were purchased and tested in the primary assay (DSF). **Hit rate 7%.**

Optimization process

- Initial SAR, built from commercially available analogues, identified a preferred series of compounds.
- Several rounds of design-synthesis-testing led to proprietary compounds series.
- Most promising compounds were further characterized in biological assays.

RESULTS

Primary Screening

GT1 stabilizes GCase

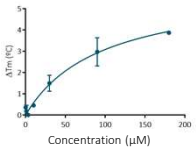

Figure 2. Dose-response effect of GT1 in GCase stabilization as measured by Differential Scanning Fluorimetry (DSF). GCase was incubated with or without increasing concentrations of GT1 and SYPRO Orange. Through a systematic increase in temperature and monitoring of SYPRO Orange emission, thermal denaturation of the protein is examined. Melting temperature shift (ΔTm) values are represented in the graph.

Non inhibitory action

GT1 is not a competitive inhibitor of GCase protein

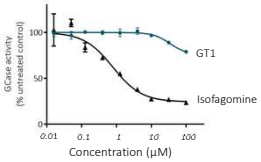

Figure 3. Inhibitory GCase activity as a function of GT1 and isofagomine concentration. Lysates from wild-type human fibroblasts were treated at different concentrations of GT1 or isofagomine, a substrate-competitive pharmacological chaperone. GCase activity was measured with the fluorogenic substrate 4-methylumbelliferyl-β-D-glucopyranoside.

Fibroblasts-based assays

GT1 enhances GCase activity and reduces GlcCer accumulation in Gaucher-patient fibroblasts

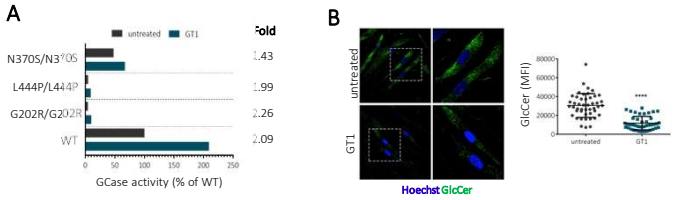

Figure 4. Enzyme enhancement activity assay and GlcCer accumulation in patient fibroblasts. (A) Gaucher-patient fibroblasts carrying the indicated mutations and wild-type fibroblasts were treated for 4 days with 25 μM of GT1. GCase activity was measured with 4-methylumbelliferyl-β-D-glucopyranoside. (B) Gaucher-patient fibroblasts carrying the p.L444P/p.L444P mutation were treated with 25 μM of GT1 for 4 days. Cells were stained with anti-GlcCer antibody and nuclei were counterstained with Hoechst reagent. Representative overlaid images are shown. Glucosylceramide MFI of >30 independent cells was quantified.

GT1 increases GCase activity and reduces phosphorylated α-synuclein levels in a differentiated dopaminergic-like neuronal model

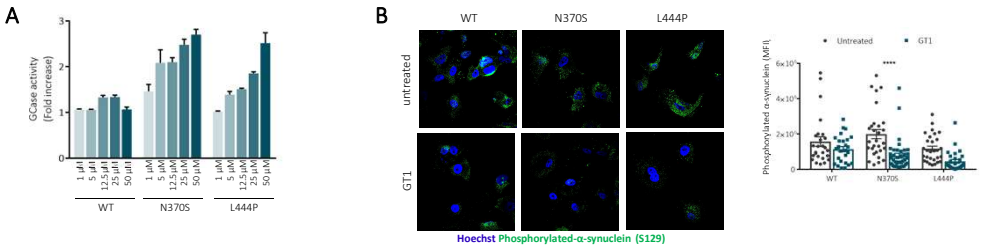

Figure 5. Enzyme enhancement activity assay and phosphorylated α-synuclein levels quantification in differentiated neuronal cell lines treated with GT1. (A) Differentiated dopaminergic neurons BE(2)-M17 carrying either wild-type or mutant N370S or L444P GBA1 mutations were treated for 4 days with the indicated concentrations of GT1. GCase activity was measured with 4-methylumbelliferyl-β-D-glucopyranoside. (B) Same cells as described in A were treated for 15 days with 25 μM of GT1. Cells were stained with anti-phosphorylated-α-synuclein (S129) antibody and nuclei were counterstained with Hoechst reagent. Representative overlaid images are shown. Phosphorylated α-synuclein MFI of between 30 and 40 individual cells for each condition was quantified.

GT-33, an improved compound, reduces α-synuclein accumulation

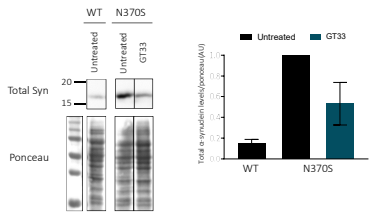

Figure 6. α-synuclein levels quantification in differentiated neuronal cell lines treated with GT33. Differentiated dopaminergic neurons BE(2)-M17 carrying mutant N370S GBA1 mutation were treated for 10 days with 12.5 μM of GT33. Cell lysates were subjected to SDS-PAGE and subsequent Western blot analysis using a synuclein mAb, followed by anti-mouse IgG-horseradish peroxidase. Ponceau was used as loading control. Molecular masses are indicated.

CONCLUSIONS

- SEE-Tx is a fast and cost-effective solution to identify structurally targeted allosteric regulators.
- We have identified novel series of non-competitive pharmacological chaperones for GCase enzyme. They are drug-like, non-competitive with natural substrate and brain penetrant, offering excellent therapeutic opportunities.
- The allosteric GCase chaperone GT1 shows the following biological actions: (i) stabilizes GCase, (ii) increases GCase activity and reduces GlcCer accumulation in patient-fibroblasts cell lines, (iii) increases GCase activity and reduces phosphorylated α-synuclein levels in differentiated dopaminergic-like neuronal model. GT33, an improved compound, reduces α-synuclein accumulation.

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