Non-competitive pharmacological chaperones for treating GBA-related diseases

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INTRODUCTION

In the field of Lysosomal storage diseases (LSDs), there is an unmet need of small-molecule drugs that can ameliorate the patient condition and the neurological manifestations in particular. Pharmacological Chaperones (PCs) are small-molecules that attack the root of the disease by stabilizing the defective enzyme, avoiding its degradation and recovering its enzymatic activity. Among them, allosteric non-inhibitory PCs offer advantages when compared with competitive, normally sugar-like, PCs by not competing with the natural substrate and potentially showing better drug-like properties, especially for CNS indications.

GBA1 gene encodes the lysosomal enzyme GCase involved in the metabolism of sphingolipids. Pathological mutations in both alleles of the GBA1 gene produce deficient GCase activity which leads to lysosomal accumulation of its substrate glucosylceramide. Moreover, mutant GBA1 heterozygous carriers are in higher risk to develop α-synucleopathies, such as Parkinson's disease. Augmentation of GCase activity would be a therapeutic strategy for Gaucher disease and α-synucleopathies patients.

The company Gain Therapeutics has implemented an innovative proprietary platform, Site-directed Enzyme Enhancement Therapy (SEE-Tx), to discover new pharmacological chaperones capable of restoring protein function.

OBJECTIVE

To develop novel allosteric GCase chaperones for the treatment of Gaucher disease and other GBA associated pathologies.

DISCOVERY STRATEGY

Gain's SEE-Tx technology was applied to identify and exploit druggable allosteric sites different from the active-site.

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.

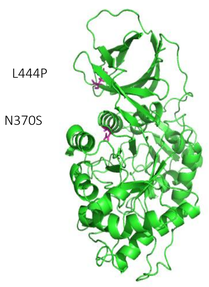

Figure 1. GCase monomer 3D structure.

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 on the DSF assay. **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 series of compounds.
- Most promising compounds were further characterized in biological assays.

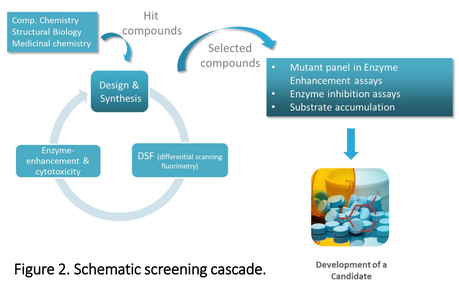

Figure 2. Schematic screening cascade.

A set of allosteric GCase chaperones were selected for their ability to increase GCase activity. A representative compound, called GT1, was extensively studied and the preliminary positive results are shown here.

CONCLUSIONS

- SEE-Tx is a fast and cost-effective solution to identify non-competitive pharmacological chaperones.
- We have identified novel series of non-competitive pharmacological chaperones for GCase enzyme. They are drug-like, non-competitive with natural substrate and potentially brain penetrant, offering excellent therapeutic opportunities.
- The allosteric GCase chaperone GT1 shows the following biological actions: (i) increases GCase activity, (ii) reduces substrate accumulation in fibroblasts cell lines, (iii) increases GCase expression levels, (iv) reduces cell death in differentiated neuronal cell-lines, and (v) reduces toxic synuclein species levels.

ACKNOWLEDGMENTS

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RESULTS

GT1, a representative compound

Primary Screening

GT1 stabilizes GCase

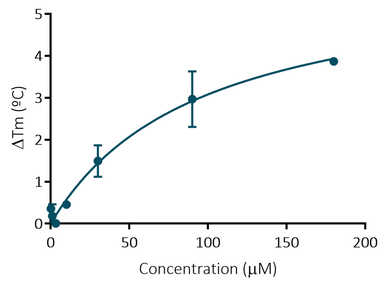

Figure 3. Dose-response effect of GT1 in GCase stabilization as measured by Differential Scanning Fluorimetry (DSF). GCase was incubated with or without increasing concentrations of GT1 and SYPRO Orange. Through a systematic increase in temperature and monitoring of SYPRO Orange emission, thermal denaturation of the protein is examined. Melting temperature shift (ΔTm) values are represented in the graph.

Enzyme inhibition

GT1 is not a competitive inhibitor of GCase protein.

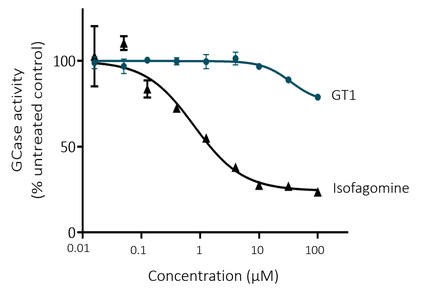

Figure 4. Inhibitory GCase activity as a function of GT1 and isofagomine concentration. Lysates from wild-type human fibroblasts were treated at different concentrations of GT1 or isofagomine, a substrate-competitive pharmacological chaperone. GCase activity was measured with the fluorogenic substrate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

Cell-based assays

GT1 enhances GCase activity in Gaucher-patient fibroblasts

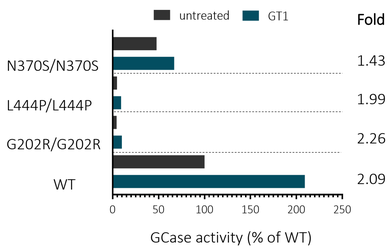

Figure 5. Enzyme enhancement activity assay in fibroblasts. Gaucher-patient fibroblasts carrying the indicated mutations and wild-type fibroblasts were treated for 4 days with 25 μM of GT1. GCase activity was measured with 4-methylumbelliferyl-β-D-glucopyranoside.

GT1 reduces GlcCer accumulation in Gaucher-patient fibroblasts

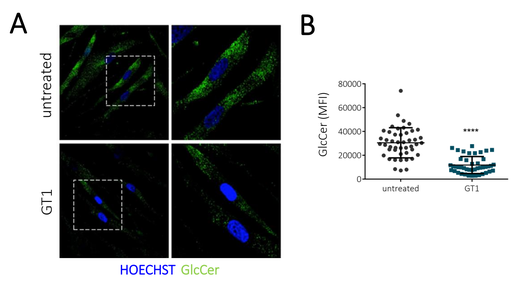

Figure 6. GlcCer accumulation in patient fibroblasts. Fibroblasts carrying the p.L444P/p.L444P mutation were treated with 25 μM of GT1 for 4 days. Cells were stained with anti-GlcCer antibody and nuclei were counterstained with Hoechst reagent. Representative overlaid images are shown (A). Glucosylceramide MFI of >30 independent cells was quantified (B).

GT1 increases GCase activity and GCase expression levels

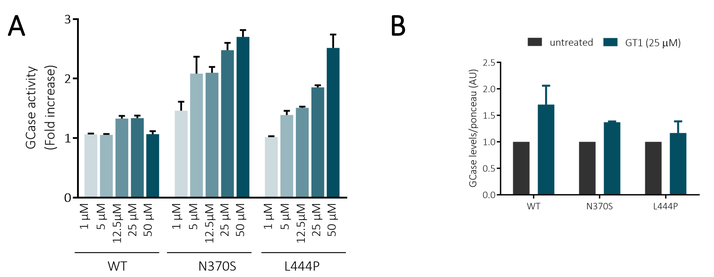

Figure 7. Enzyme enhancement activity assay and GCase levels quantification in differentiated neuronal cell lines. Differentiated dopaminergic neurons BE(2)-M17 carrying either wild-type or mutant N370S or L444P GBA1 mutations were treated for 4 days (A) or 15 days with the indicated concentrations of GT1 (B). GCase activity was measured with 4-methylumbelliferyl-β-D-glucopyranoside (A) and GCase levels were quantified by western blot (B).

GT1 reduces cell death in differentiated neuronal cell lines

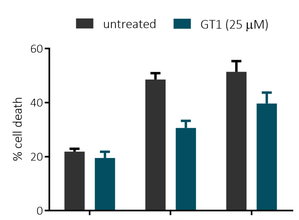

Figure 8. Cell death quantification. Differentiated dopaminergic neurons BE(2)-M17 carrying either wild-type or mutant N370S or L444P GBA1 mutations were treated for 15 days with 25 μM of GT1. Cell death was measured by Muse® Count&Viability Assay Kit (Merck) following the manufacturer's instructions.

GT1 reduces toxic α-synuclein species levels in differentiated neuronal cell lines

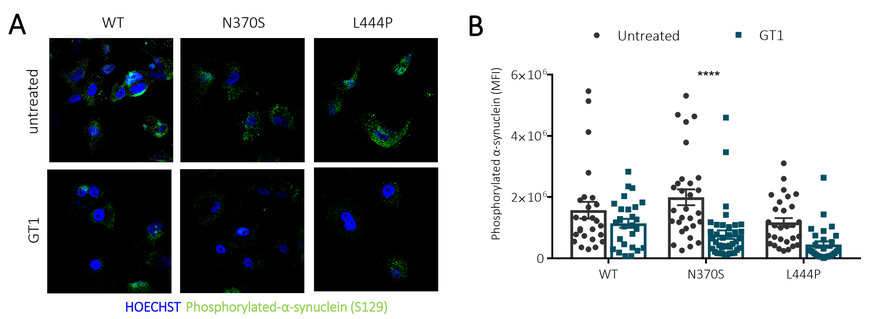

Figure 9. Phosphorylated α-synuclein levels quantification. Differentiated dopaminergic neurons BE(2)-M17 carrying either wild-type or mutant N370S or L444P GBA1 mutations were treated for 15 days with 25 μM of GT1. Cells were stained with anti-phosphorylated-α-synuclein (S129) antibody and nuclei were counterstained with Hoechst reagent. Representative overlaid images are shown (A). Phosphorylated-α-synuclein MFI of between 30 and 40 individual cells for each condition was quantified (B).