Preclinical Development of an Oral Small Molecule That Increases GCase Enzyme Activity for the Treatment of Gaucher Disease

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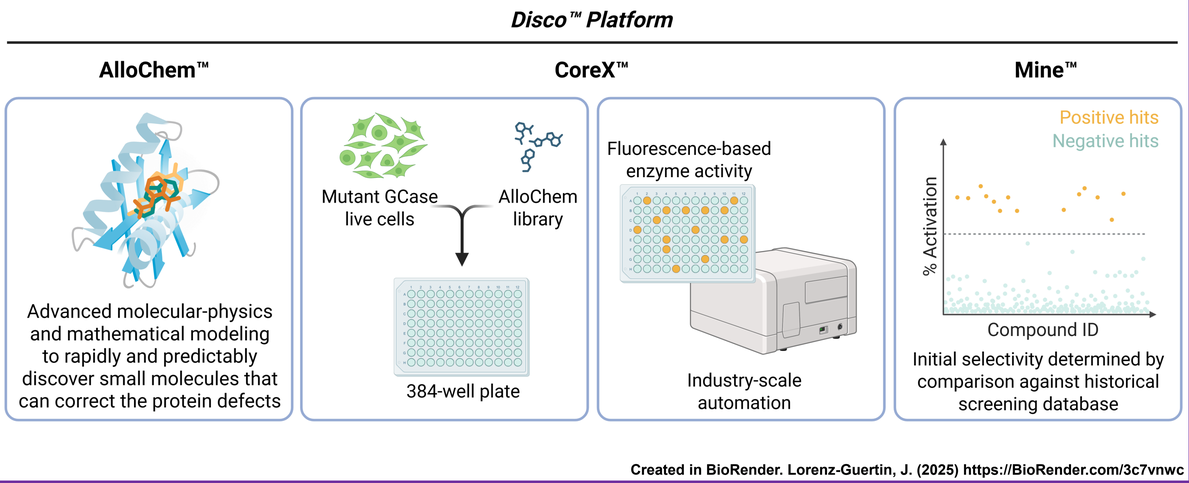
Background and Approach

Gaucher disease is caused by mutations of the *GBA1* gene and subsequent loss of activity in the enzyme GCase, causing harmful accumulation of the lipids glucosylceramide (GlcCer) and glucosylsphingosine (GlcSph). Gaucher patients are primarily treated by bi-weekly recombinant enzyme replacement therapy (ERT) via intravenous infusions. ERT treatments have known deficiencies including limited CNS penetration, potential autoimmune response and patient time burden. One therapeutic strategy to overcome these issues is to identify an orally-available small molecule capable of restoring enzyme function in mutated forms of GCase. To address this, we leveraged our Disco™ platform to rapidly and predictably discover novel small molecules that can correct protein defects. Hit molecules were determined by their ability to elevate enzymatic activity in cells expressing mutated GCase. Medicinal chemistry was used for structure optimization to improve potency, stability, and overall properties of the series.

Optimization and profiling efforts were performed in multiple species, confirmed in cell-free recombinant GCase and in Gaucher patient fibroblasts. Further, this series significantly reduced levels of GlcCer and GlcSph in patient fibroblasts by lipidomics. In vivo, we observe a dose-dependent increase in plasma drug concentration via intraperitoneal and oral administration. Finally, in a conduritol-β-epoxide (CBE)-induced Gaucher mouse model, administration of our compound significantly reduced GlcCer and GlcSph levels peripherally and in the brain of mice. Sharp Therapeutics has discovered a novel series of small molecules that show favorable drug-like characteristics in vitro and in vivo and are well-suited to be clinically evaluated for the treatment of Gaucher disease.



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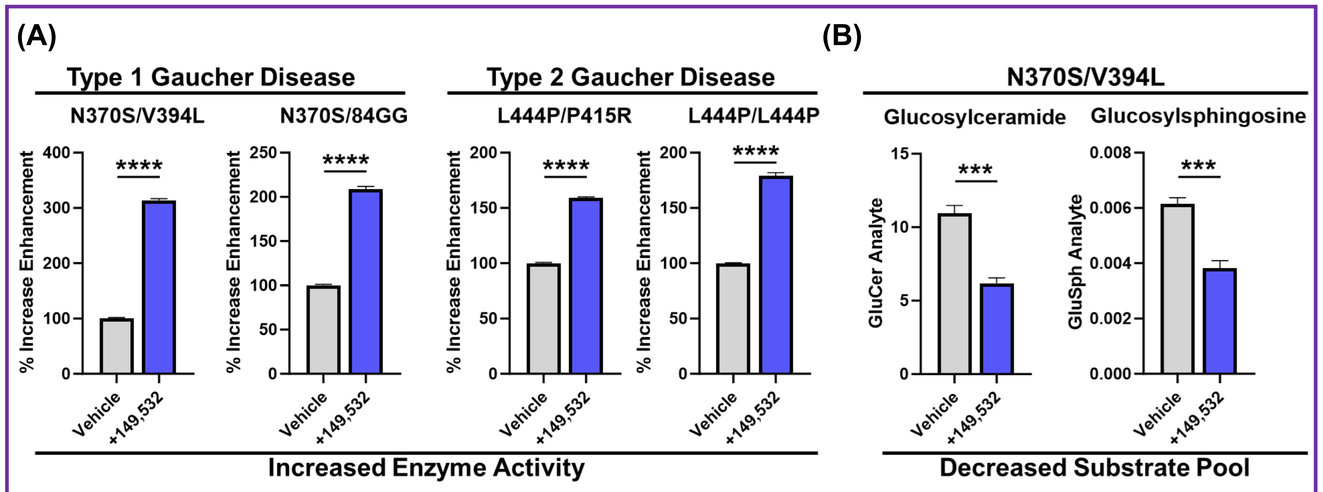

Figure 2: Enzyme Activation and Reduced Lipids in Patient Fibroblasts. Gaucher patient fibroblasts were treated with ’532 and GCase activity was measured using 4-MUG (*n*=8/condition). N370S/V394L mutant patient fibroblasts were treated with ’532 for 10 days and subjected to lipidomic analysis to measure total GlcCer and GlcSph (d18:1) substrate analyte relative to total protein concentration. 20.0 μM testing concentration (*n*=4/condition). ***p<0.001, ****p<0.0001*

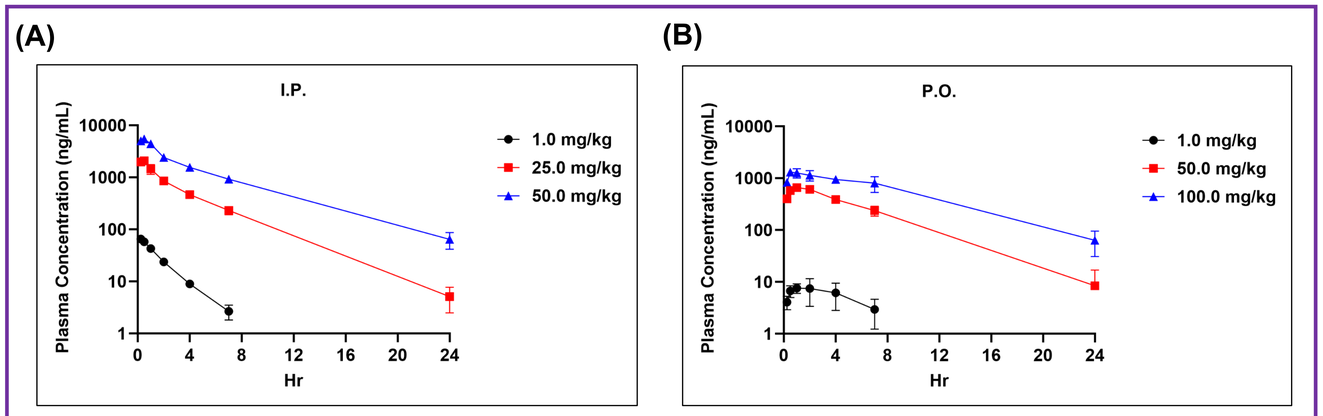

Figure 3: ’532 Pharmacokinetics. (A) Mice injected I.P. with ’532 plasma drug exposure and a respective t½ range of 1.5-4.3 hours. **(B)** P.O. administration of ’532 oral drug absorption and plasma concentration of compound with a t½ range of 2.0-7.0 hours. *n*=3/arm in both experiments.

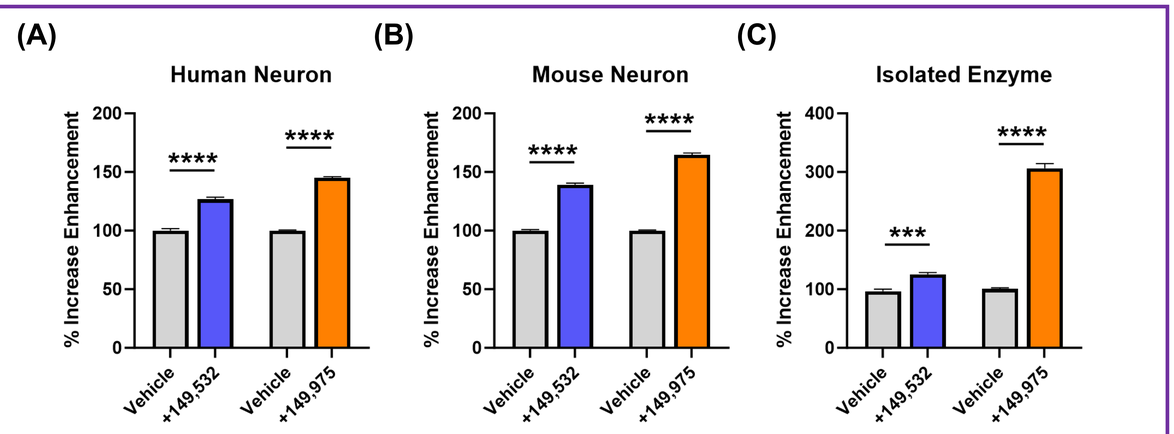

Figure 1: Lead Series Compounds Enhance GCase Activity. A. Human SH-SY5Y and **B.** Mouse N2a cells were treated with 10.0 μM ’532 or ’975 and measured for GCase activity using fluorescent 4-MUG substrate. (*n*=8/condition) **C.** Human recombinant GCase enzyme treated with 20.0 μM compound and enzyme activity was measured by 4-MUG (*n*=4/condition). Normalized to DMSO vehicle in all assays. ***p<0.001, ****p<0.0001*

(A)

Glucosylceramide (GlcCer)				
Compound:	’975 + CBE	’976 + CBE	’901 + CBE	’927 + CBE
vs. CBE Control	p ≤ 0.05	p ≤ 0.05	p ≤ 0.05	p ≤ 0.05
Plasma	✓	✓	✓	✓
Liver	✓	✓	✓	✓
Spleen	⊗	⊗	⊗	⊗
Brain	⊗	✓	✓	✓

(B)

Glucosylsphingosine (GlcSph)				
Compound:	’975 + CBE	’976 + CBE	’901 + CBE	’927 + CBE
vs. CBE Control	p ≤ 0.05	p ≤ 0.05	p ≤ 0.05	p ≤ 0.05
Plasma	⊗	✓	✓	✓
Liver	⊗	⊗	✓	✓
Spleen	⊗	⊗	✓	✓
Brain	⊗	✓	✓	✓

Figure 4: Reduced Lipids in a CBE Mouse Model. Mice were injected with CBE 100mg/kg I.P. once-daily for five days to induce GlcCer and GlcSph accumulation. SEL compounds were co-administered at 75mg/kg I.P. **(A)** GlcCer and **(B)** GlcSph levels were analyzed by LC-MS/MS (*n*=7-10/condition). Student’s t-test statistical comparison vs. CBE only control mice.

Conclusions

- Our lead structural series is active in multiple species expressing wild-type GCase
- ’532 enhances GCase activity in patient fibroblasts and reduces lipid accumulation
- ’532 has favorable pharmacokinetic properties and oral bioavailability
- This series reduces mouse GlcCer and GlcSph peripherally and in the brain

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