# DISCOVERY OF SMALL MOLECULES FOR THE TREATMENT OF PD-GBA

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## Background and Approach:

Heterozygous mutations in the *GBA1* gene, encoding for the lysosomal enzyme glucocerebrosidase (GCase), are the strongest genetic risk factor for Parkinson's disease (PD). Furthermore, GCase enzymatic activity is reduced in PD-*GBA1* patients, suggesting that loss of GCase activity contributes to the pathogenesis of Parkinson's disease. Therefore, therapeutics targeted at increasing the activity of GCase may prove efficacious in the treatment of PD. Saposin C, a well-characterized co-factor for GCase activity, is a lysosomal cleavage product of its precursor, prosaposin (PSAP). We previously discovered novel small molecules that increase progranulin (PGRN) as a potential treatment for frontotemporal dementia, and given the interrelationship between PGRN and PSAP in lysosomal biology, we interrogated the effect of these molecules on PSAP.

Here we report the characterization of one of these molecules which promotes increases in secretion and intracellular expression of PSAP, along with increases in intracellular saposins. Using a live-cell GCase activity assay, we also show this compound leads to a concentration-dependent increase in endogenous GCase enzymatic activity in WT cells. Furthermore, our compound can increase GCase activity in N370S PD-*GBA1* mutant lymphoblasts. The increase in saposin C expression, through increased PSAP production, or a combination of both is consistent with the observed downstream increased GCase activity. In conclusion, we have discovered small molecules that increase WT GCase activity which can be developed further as potential therapeutics for Parkinson's disease.

**Figure 1. Compound 2 increases levels of PSAP and saposins.**
**(A)** Secreted PSAP from BV-2 cells was measured by ELISA. **(B)** Intracellular PSAP and saposins were measured by WB in iPSC-derived neurons.

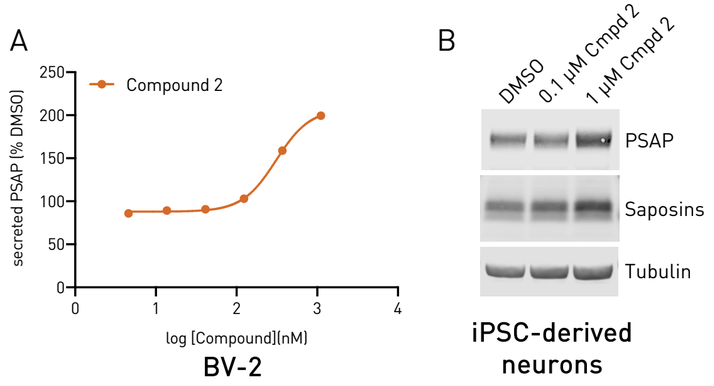
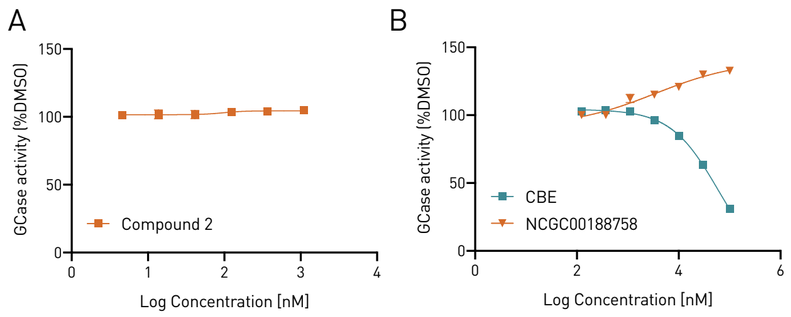



**Figure 2. Compound 2 is not a direct activator of GCase.**
**(A)** GCase activity was measured *in vitro* using recombinant hGBA and the substrate 4MU-β-Glc in the presence of Compound 2 and **(B)** known GCase modulators.



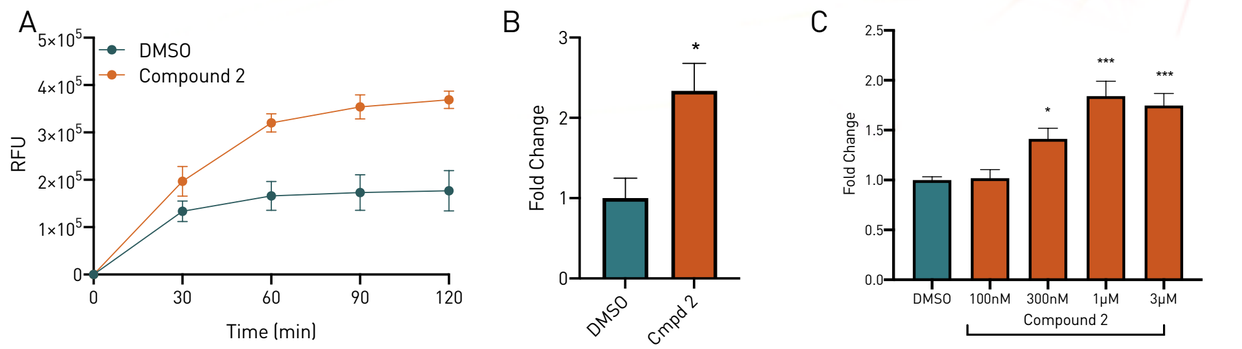




**Figure 3. Compound 2 increases lysosomal GCase activity in a live-cell activity assay.** Enzyme activity was measured in HeLa cells with the fluorescent GCase substrate PFB-FDGlu after treatment with Compound 2. BafA was used to determine lysosomal-specific enzyme activity. **(A)** Time course of *in situ* activity in cells treated with 1µM Compound 2. **(B)** Fold change of the AUC of treated cells over DMSO. **(C)** Compound 2 concentration-response change of lysosomal GCase activity. *p<0.05, **p<0.01, ***p<0.001

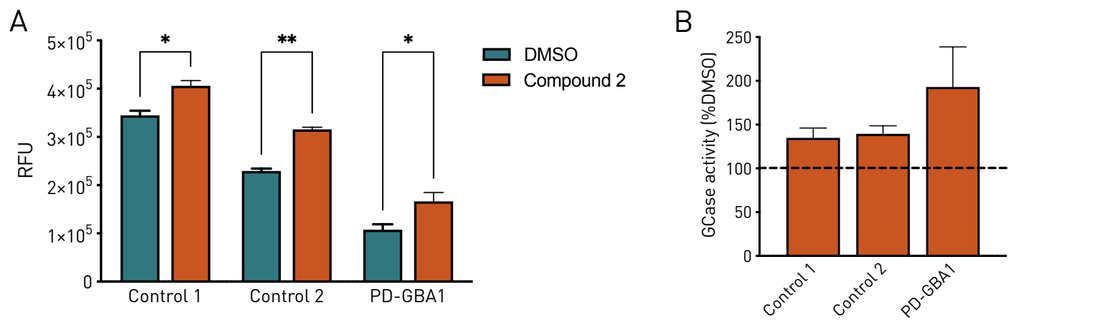



**Figure 4. Compound 2 increases GCase activity in PD-*GBA1* patient-derived lymphocytes. (A)** GCase activity was measured *in vitro* in B-lymphocytes from PD-*GBA1* patients harboring the N370S mutation and age-matched controls using the substrate 4MU-β-Glc after 24 hr treatment with 1µM Compound 2 (representative data). **(B)** Increase in GCase enzymatic activity in each cell line with treatment (average of multiple experiments). *p<0.05, **p<0.01

## Conclusions:

- Small molecules were discovered that increase intracellular PSAP and saposins, along with secretion of PSAP
- Compound 2 increases GCase enzyme activity in a concentration-dependent manner in HeLa cells using a live-cell assay
- Compound 2 increases GCase activity in PD-*GBA1* N370S mutant patient-derived B-lymphocytes
- More studies are ongoing to understand the precise mechanism by which these compounds mediate GCase activity increase

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