Targeting glucocerebrosidase with structurally targeted allosteric regulators corrects abnormal phenotypes in models of Parkinson's disease

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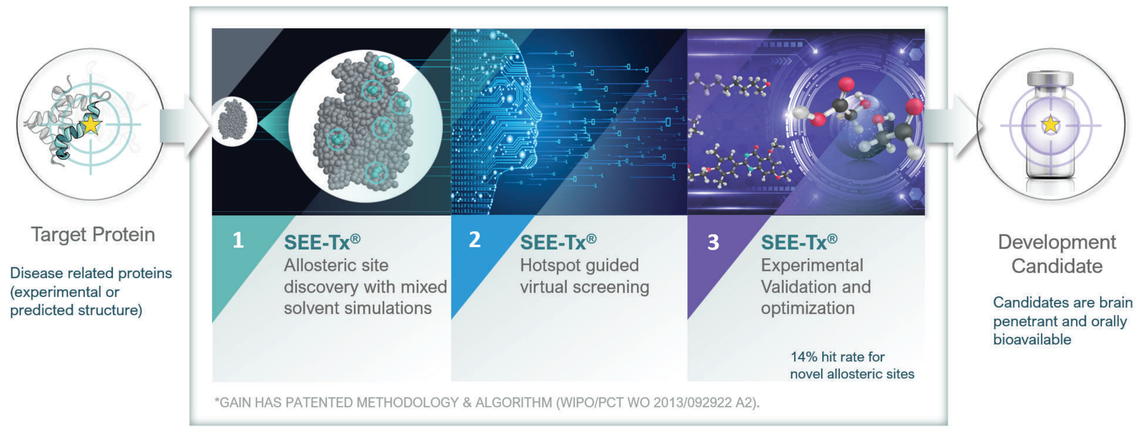
Introduction

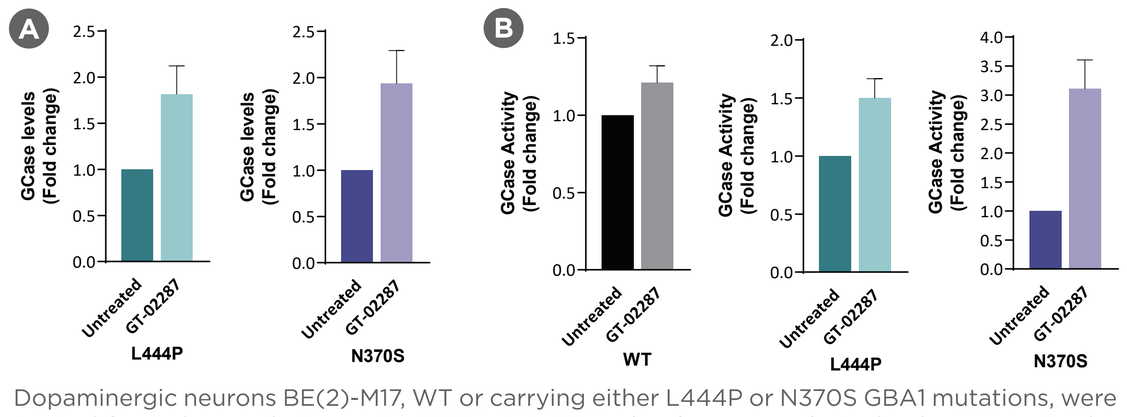
Mutations in the *GBA1* gene encoding the acid β-glucocerebrosidase (GCase) represent the most common genetic risk factor for Parkinson's disease (PD). The hallmark of PD is the presence of alpha-synuclein (α-syn) accumulation in specific areas of the brain. Interestingly, there appears to be an inverse relationship between GCase and α-syn levels: reduced GCase function is associated with increased α-syn accumulation as well as a change from its soluble form to its aggregated form, and it has been postulated that α-syn accumulation may reduce overall GCase activity. For these reasons, decreased GCase activity and levels may contribute to PD pathogenesis and restoring dysfunctional GCase may therefore represent a potential therapeutic strategy.

SEE-Tx™ Technology



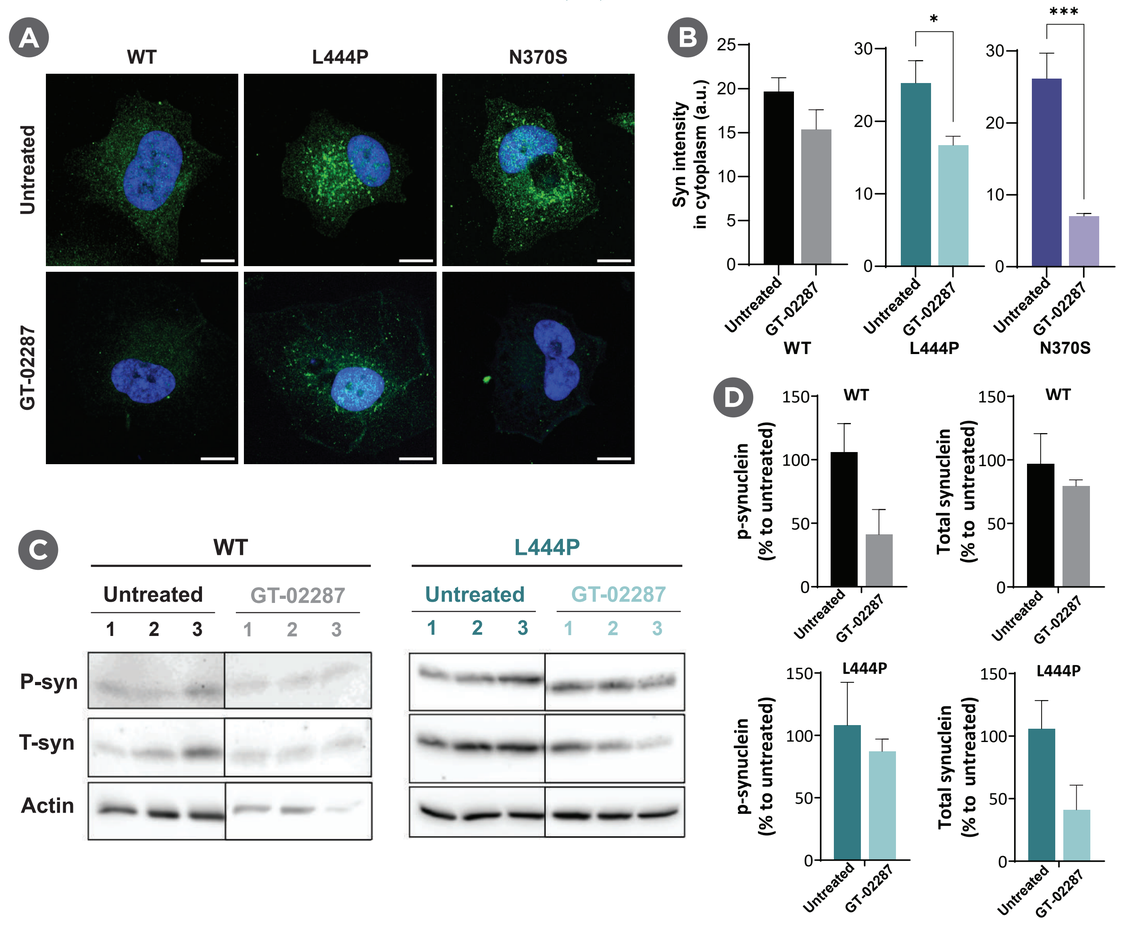
Aim: Restore GCase function using allosteric regulators to slow or stop PD progression

1 GT-02287 increases GCase levels and activity in a dopaminergic neuronal model



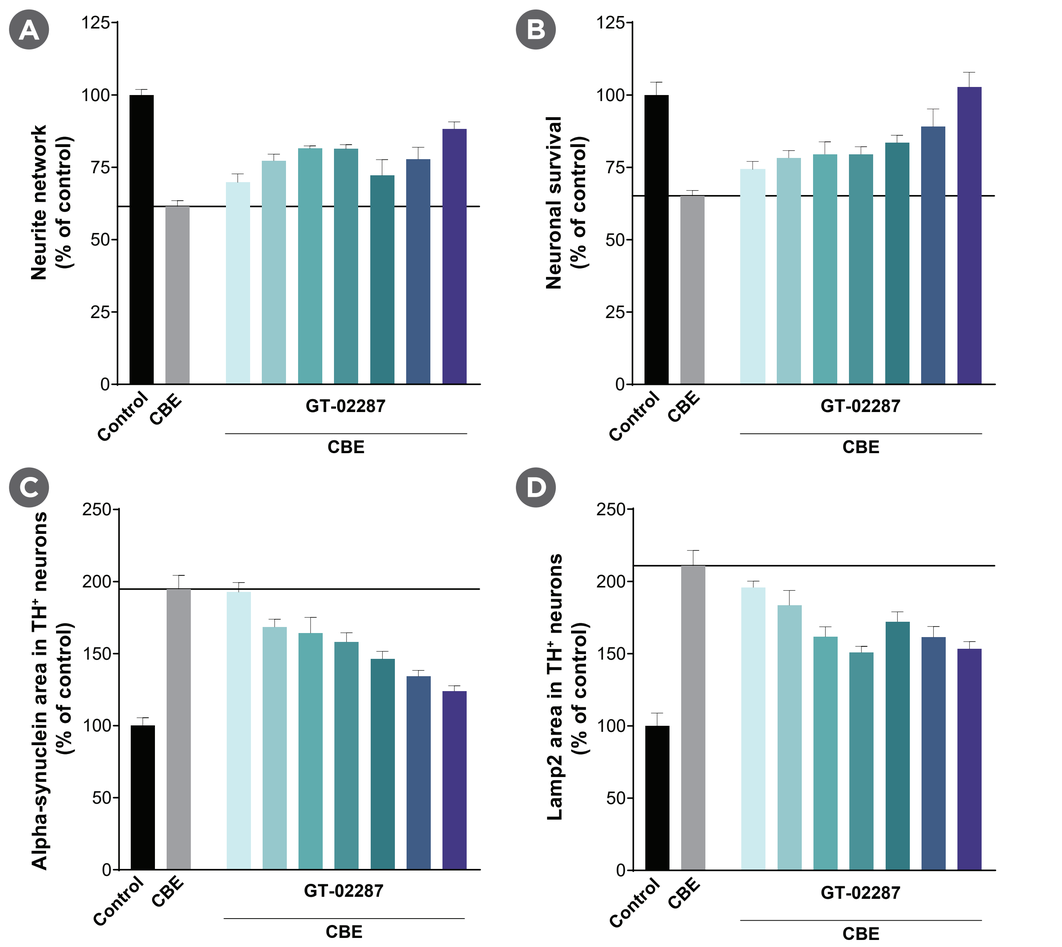
Dopaminergic neurons BE(2)-M17, WT or carrying either L444P or N370S GBA1 mutations, were treated for 4 days with 25 μM GT-02287. (**A**) GCase levels were evaluated in homogenates by western blot and normalized by Ponceau. (**B**) GCase activity was measured using 4-methylumbelliferyl-β-D-glucopyranoside and normalized to untreated.
Results are presented as mean+SEM from 3 independent experiments.

2 GT-02287 reduces alpha-synuclein levels in WT, L444P and N370S BE(2)-M17 cells



Dopaminergic neurons BE(2)-M17, WT or carrying either N370S or L444P GBA1 mutations, were treated for 10 days with 25μM GT-02287. (**A**) Cells were stained with anti-α-synuclein oligomer specific (Syn33) antibody (green) and nuclei were counterstained with DAPI (blue). Representative overlay images are shown, Scale bars: 10μm. (**B**) Syn intensity inside the cytoplasm was measured. (**C**) Typical WB of phosphorylated α-syn (p-syn) and total synuclein (T-syn). (**D**) Quantification of α-syn and phosphorylated α-syn (Ser 129 Ab).
Results are presented as mean+SEM. One-way ANOVA (Welch correction) were used comparing each column with its corresponding untreated. Significance is denoted: *p<0.5, ***p<0.001

3 GT-02287 is neuroprotective and improves lysosomal health as well as synuclein pathology



Primary cultures of rat mesencephalic neurons were established. On day 6, GT-02287 was applied and after 24 hours, CBE (400 μM) was added to the culture medium for 48 hours. On day 8, the culture was fixed and stained for tyrosine hydroxylase (TH), a marker for dopaminergic neurons. (**A**) Neurite network, (**B**) neuronal survival, (**C**) aggregated synuclein and (**D**) lysosomal area were evaluated.

Conclusions

SEE-Tx™ is a fast and cost-effective solution that has allowed us to identify structurally targeted allosteric regulators (STARs) of the GCase enzyme that are orally bioavailable and brain-penetrant.

GT-02287:
- **Enhances GCase levels and activity in dopaminergic cells**
- **Effectively reduces alpha-syn in a neuronal cell model**
- **Increases neuronal viability and reduces lysosomal area and pathogenic synuclein in dopaminergic neurons**

GT-02287 restores GCase-related key biological activities found to be impaired in many forms of PD, thus warranting further development towards the clinic.

Acknowledgements: