GT-02287, a clinical stage GCase enhancer, displays neuroprotection and restores motor function in preclinical models of Parkinson's disease following delayed administration

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Objective

To investigate the ability of the clinical stage GCase enhancer, GT-02287, to protect dopaminergic neurons and to rescue motor symptoms in delayed treatment paradigms in *in vitro* and *in vivo* models of GBA1-Parkinson's disease.

Background

Bi-allelic mutations in *GBA1* encoding lysosomal enzyme β-glucocerebrosidase (GCase) result in the lysosomal storage disorder Gaucher disease. *GBA1* mutations are also the most prevalent genetic risk factor for developing Parkinson's disease (PD) and there is evidence that lysosomal GCase dysfunction occurs in idiopathic as well as genetic forms of the disease. Reduced GCase activity leads to build-up of GCase substrates, progressive lysosomal dysfunction, and impaired metabolism of α-synuclein which lead to neuroinflammation, neurodegeneration and motor deficits in PD. Gain Therapeutics applied its innovative, proprietary computational drug discovery platform to the discovery of a novel allosteric binding site on GCase and small molecules that are structurally targeted allosteric regulators (STARs) of GCase. Gain Therapeutics is developing GT-02287 which stabilizes GCase, protects it from degradation, facilitates its trafficking to the lysosome and restores its function. **GT-02287 is an orally bioavailable, brain penetrant, clinical candidate currently in a Phase 1 clinical trial in healthy volunteers.**

Methods

In *in vitro* studies, the rescue effect of GT-02287 was assessed using primary cultures of rat dopaminergic neurons injured with mild inhibition of GCase (using conduritol β-epoxide, CBE, at 20 µM) combined with the application of α-synuclein (αSyn)-preformed fibrils (PFFs) at 250 nM. Cultures were also treated with αSyn-PFFs alone. GT-02287 was applied 16h after the combined αSyn-PFFs + CBE injury or after αSyn-PFFs alone. Cultures were stained with an antibody to tyrosine hydroxylase (TH) to identify dopaminergic neurons, combined with an antibody to Lamp2, a lysosomal marker. In *in vivo* studies, mice were bilaterally injected with αSyn-PFFs into the striatum and chronic low-level (50 mg/kg i.p.) CBE was applied every other day for 27 days. GT-02287 was administrated orally once daily starting at two different timepoints: 4 days or 8 days after the initial combined toxic insult. Motor performance was assessed at day 14 and at day 27 of the study. After sacrifice (day 28), plasma levels of NfL were assessed as a marker of neurodegeneration.

Results

GT-02287 rescued cultured rat dopaminergic neurons injured with αSyn-PFFs, with or without the irreversible GCase inhibitor CBE, even when the compound was applied substantially later than the toxic insult. In the *in vivo* mouse model of GBA1-PD, GT-02287 rescued locomotor impairment even when treatment began several days after the initial toxic insult. In fact, the longer that GT-02287 was applied, the greater the improvement, suggesting a reversal of the locomotor deficit. Motor rescue was reflected in reduced levels of NfL in plasma after GT-02287 treatment.

1. GT-02287 rescues neuronal survival, neurite network and improves lysosomal pathology following delayed administration of α-syn PFFs injury with and without CBE in rat dopaminergic neurons

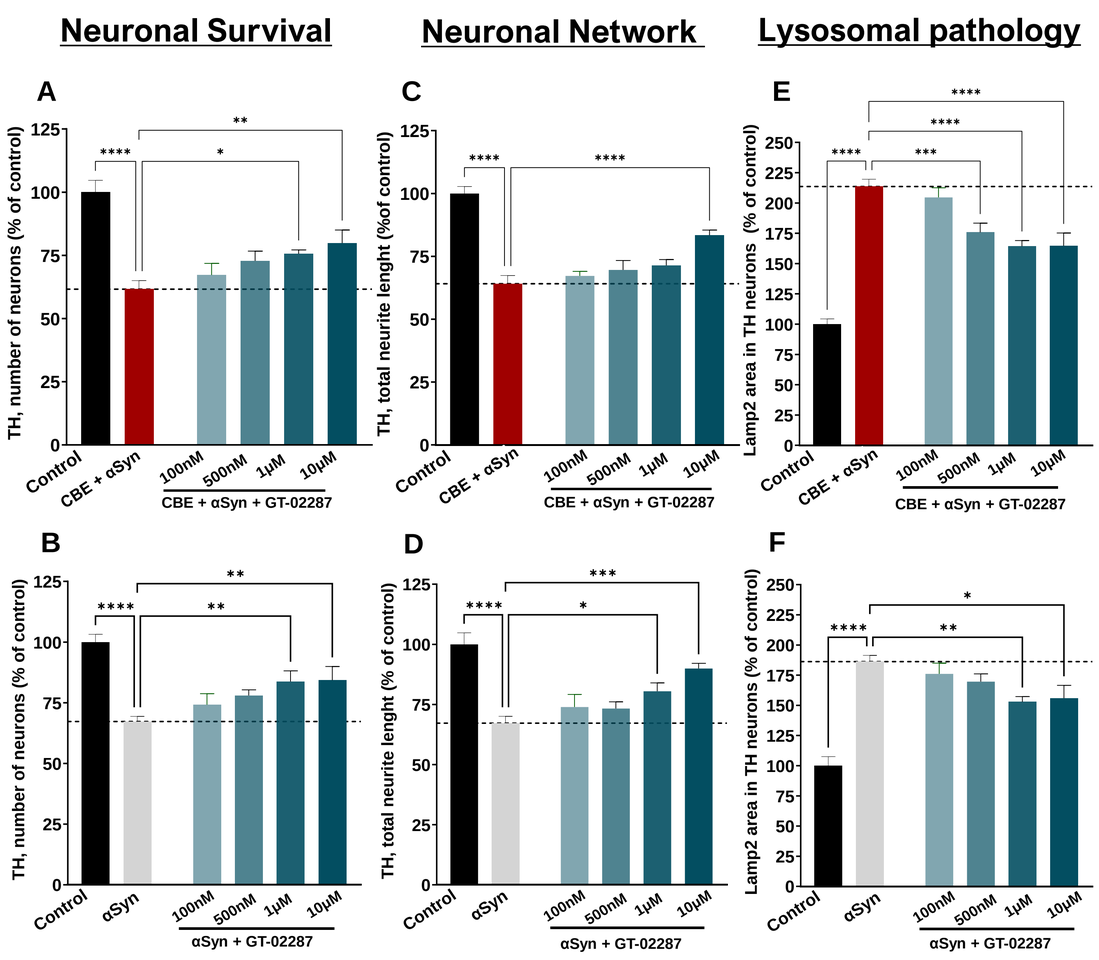
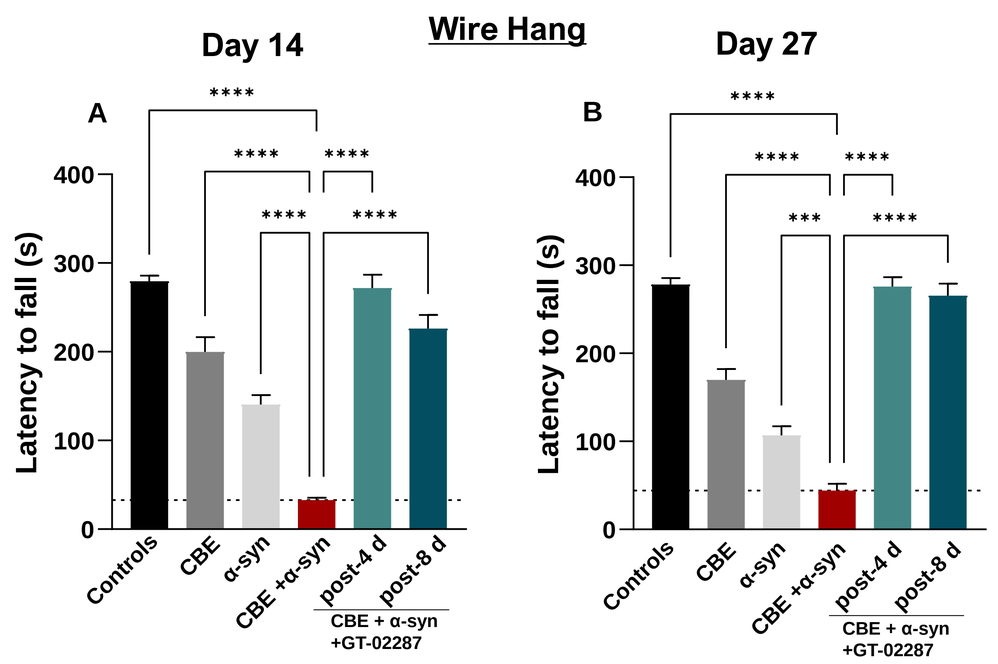


Fig.1 Neuronal survival (number of TH+ dopaminergic neurons) (A, B), neurite network (total neurite length in TH+ neurons) (C, D) and lysosomal pathology (Lamp2+ area in TH+ neurons) (E, F) in control (black bars), αSyn + CBE injured (red bars), αSyn injured (grey bars) and αSyn + CBE (A,C,E) or αSyn (B,D,F) injured cultured neurons treated with GT-02287 (100 nM, 500 nM, 1 µM and 100 µM) (green bars). *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 versus CBE + αSyn and/or αSyn; one-way ANOVA followed by Fisher's LSD test.

2. GT-02287 rescues motor impairment following delayed administration in the CBE + α-syn PFFs GBA1-PD mouse model



GT-02287 reduces plasma NfL following delayed administration in the CBE + α-syn PFFs GBA1-PD mouse model

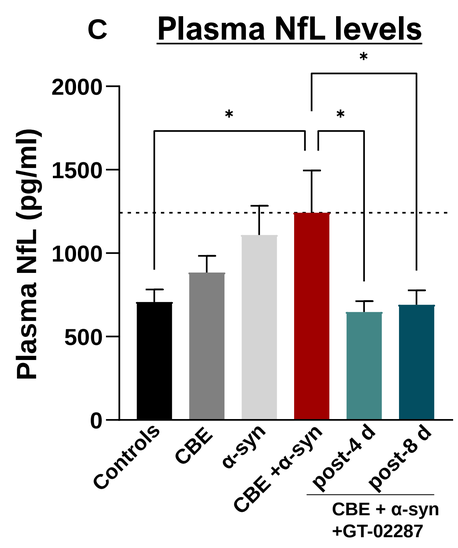


Fig.2 Motor performance (latency to fall, s) assessed by the wire hang test at day 14 (A) and at day 27 (B), as well as the plasma levels of neurofilament light chain (NfL) (C) at sacrifice (day 28) are shown in controls (black bars), CBE-injured (dark grey bars), αSyn-injured (light grey bars), CBE + αSyn-injured (red bars) and CBE + αSyn-injured mice treated with GT-02287 (90 mg/kg oral q.d.) post-4 days (light green bars) and post-8 days (dark green bars) after the initial combined toxic insult. Data is shown as mean ± S.E.M. (n=10-12), one-way ANOVA followed by Dunnett's multiple comparison test. Significant difference as compared to CBE + αSyn.*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

Conclusions

These new data support the potential of GT-02287 as a disease-modifying therapy for Parkinson's disease that is already clinically established. Plasma NfL, an emerging biomarker of neurodegeneration, was reduced to control levels, reflecting the motor deficit rescue observed in the wire hang test. GT-02287 appeared to reverse motor deficit as treatment continued.