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)-prefrmed 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 NIL 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 NIL 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**

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

**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 NIL, 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.