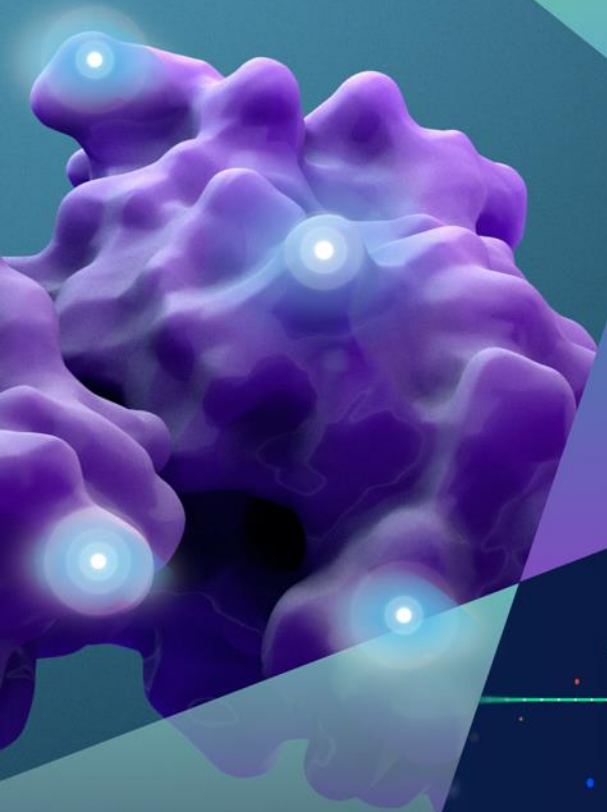
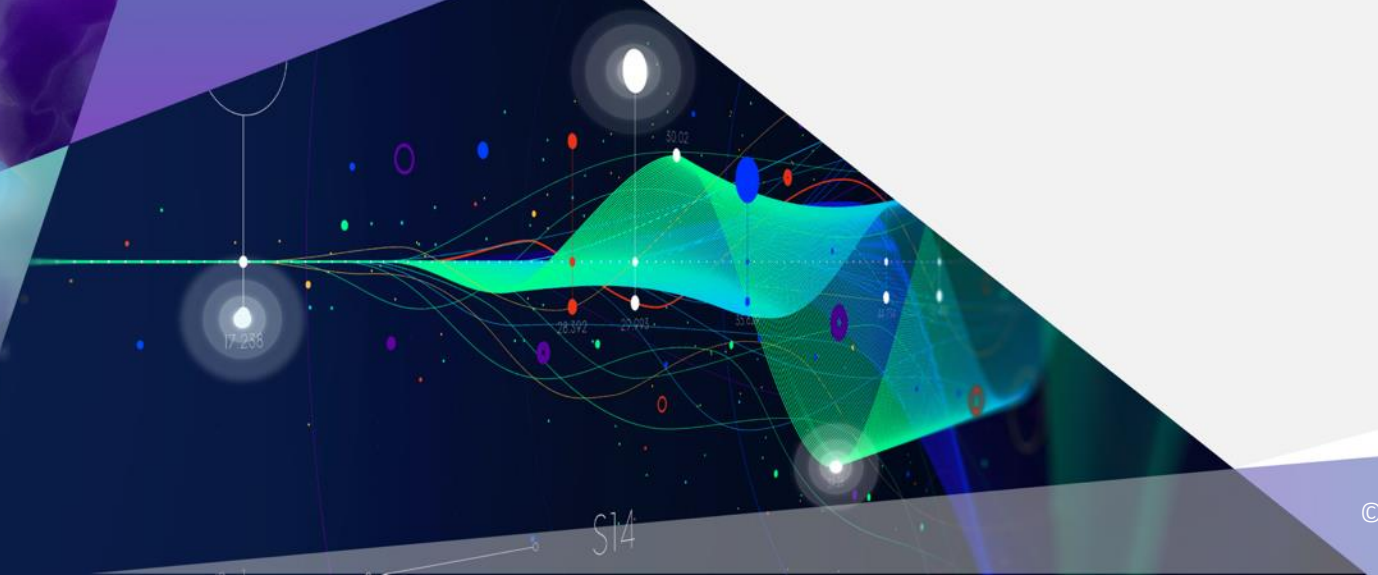
GAIN THERAPEUTICS

IAPRD
01 - 04 May 2021

WORLD CONGRESS ON PARKINSON'S DISEASE AND RELATED DISORDERS





PRECLINICAL DEVELOPMENT OF BRAIN-PENETRANT STRUCTURALLY TARGETED ALLOSTERIC REGULATORS FOR THE TREATMENT OF GBA1 PARKINSON'S DISEASE AND RELATED α-SYNUCLEOPATHIES

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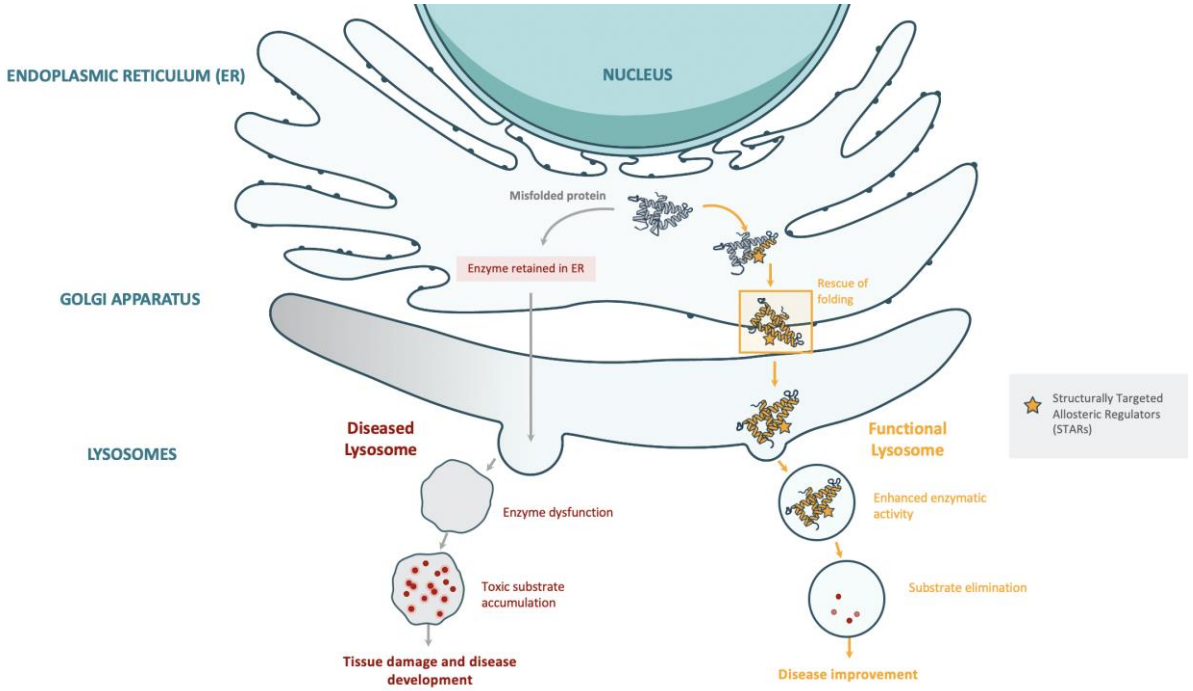
Development of STAR^{s} targeting GCase for Parkinson's Disease

We propose using structurally targeted allosteric regulators (STAR^{s}) that bind the misfolded forms of GCase trapped in the ER and enhance the processing from the ER to the lysosome, improving lysosomal GCase activity, restoring normal lysosomal/autophagic activity and ultimately decreasing α-synuclein levels.



Lead compounds **GT-02287 and GT-02329**:

- stabilize the protein and enhance GCase activity at 1-digit μM dose
- show a significant neuroprotective effect
- increase and stabilize Gcase in WT mice
- reduce toxic substrate accumulation in *in vitro* and *in vivo* PD models

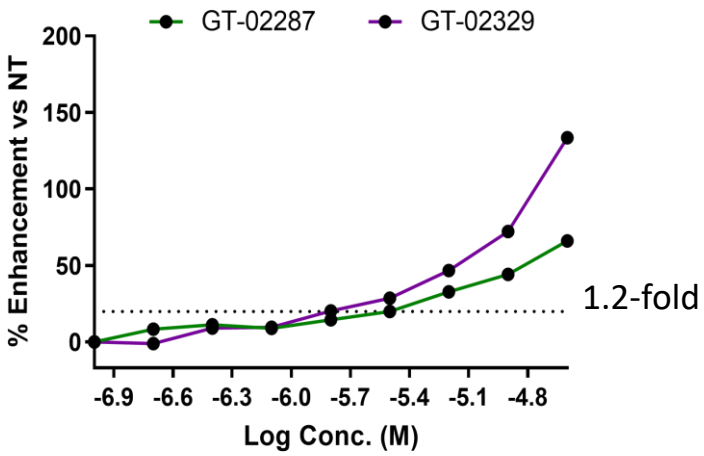
The compounds are able to restore relevant biological functions which are impaired in GBA1 synucleinopathies encouraging further development toward clinical research, particularly in GBA1 PD patients.

GT-02287 and GT-02329

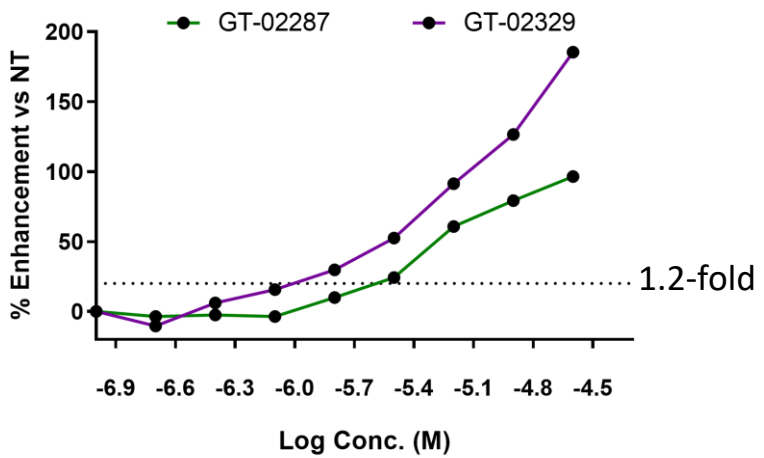
In vitro dose-response activity curves in most relevant fibroblasts

GT-02287 and GT-02329 show one-digit micromolar EC50 in WT and p.L444P/p.L444P fibroblasts.

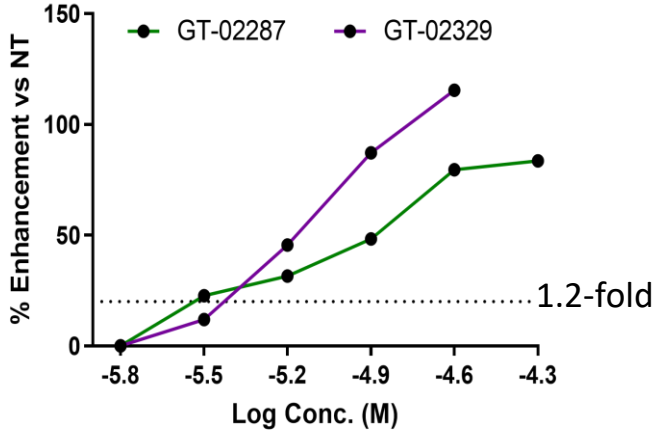
WT



p.L444P/p.L444P



p.N370S/ins

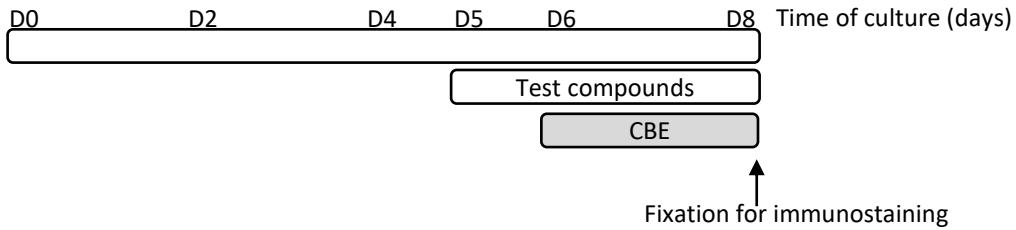


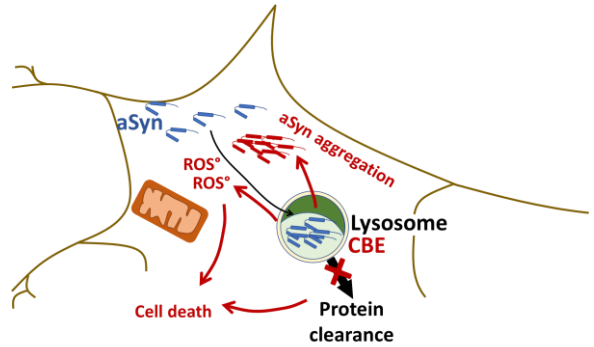
Conc. (µM)	Log Conc. (M)
50.00	-4.3
25.00	-4.6
12.50	-4.9
6.25	-5.2
3.13	-5.5
1.56	-5.8
0.78	-6.1
0.39	-6.4
0.20	-6.7
0.10	-7
0.05	-7.3
0.02	-7.6
0.01	-7.9

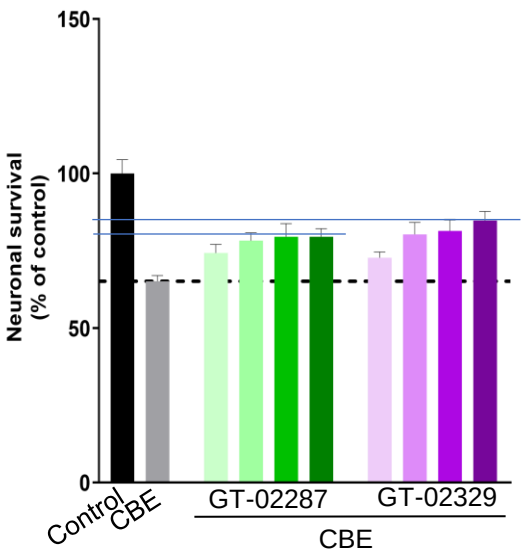
Dose-Response Assays. WT or Gaucher-patient derived fibroblasts were treated with GT-02287 and GT-02329 at different concentrations (0.2 – 25 µM). After a 4-day treatment, GCase activity was assessed using the 4-MU-β-D-glucopyranoside substrate. The assay reaction is started by the addition of 28 µL of 5 mM of 4-MU-beta-D-glucopyranoside in 0.1 M acetate buffer (pH 4) to each well. Plates are incubated at 37ºC for 1h and the reaction is stopped by the addition of 200 µL of glycine buffer (pH 10.7) to each well. Liberated 4-methylumbelliferone is measured (excitation 340 nm, emission 460 nm).

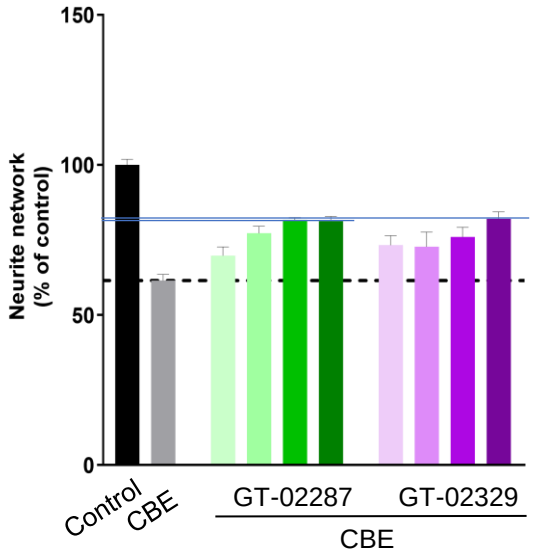
GT-02287 and GT-02329
In vitro PoC in CBE-induced pharmacological model

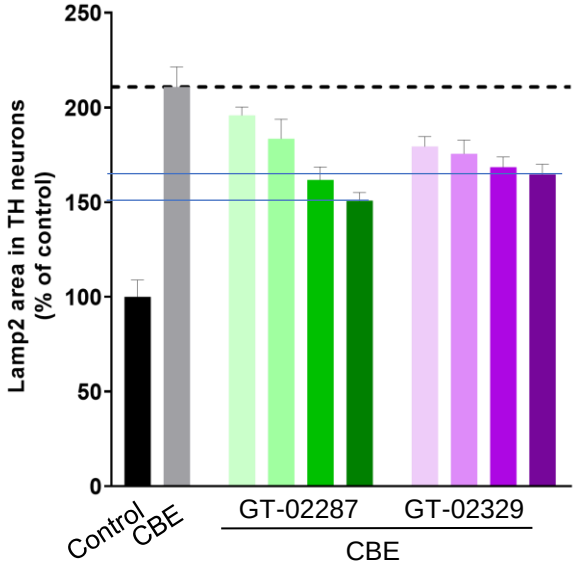
GT-02287 and GT-02329 are neuroprotective and lower lysosomal as well as synuclein pathology at nM concentrations

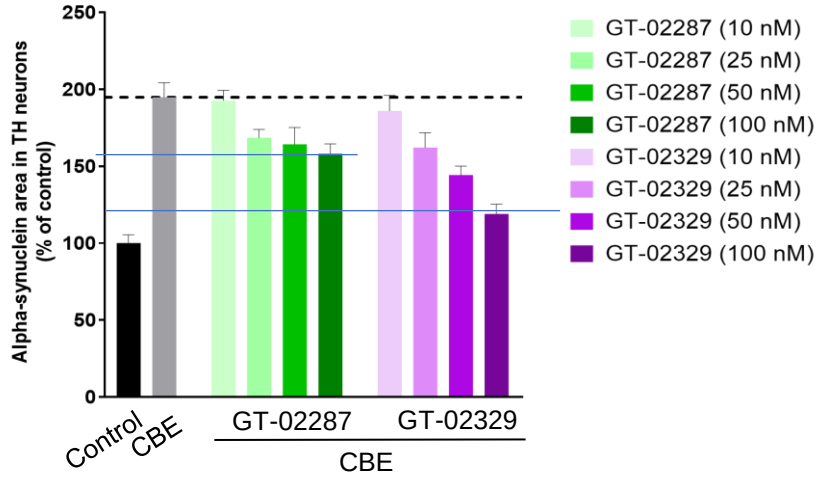








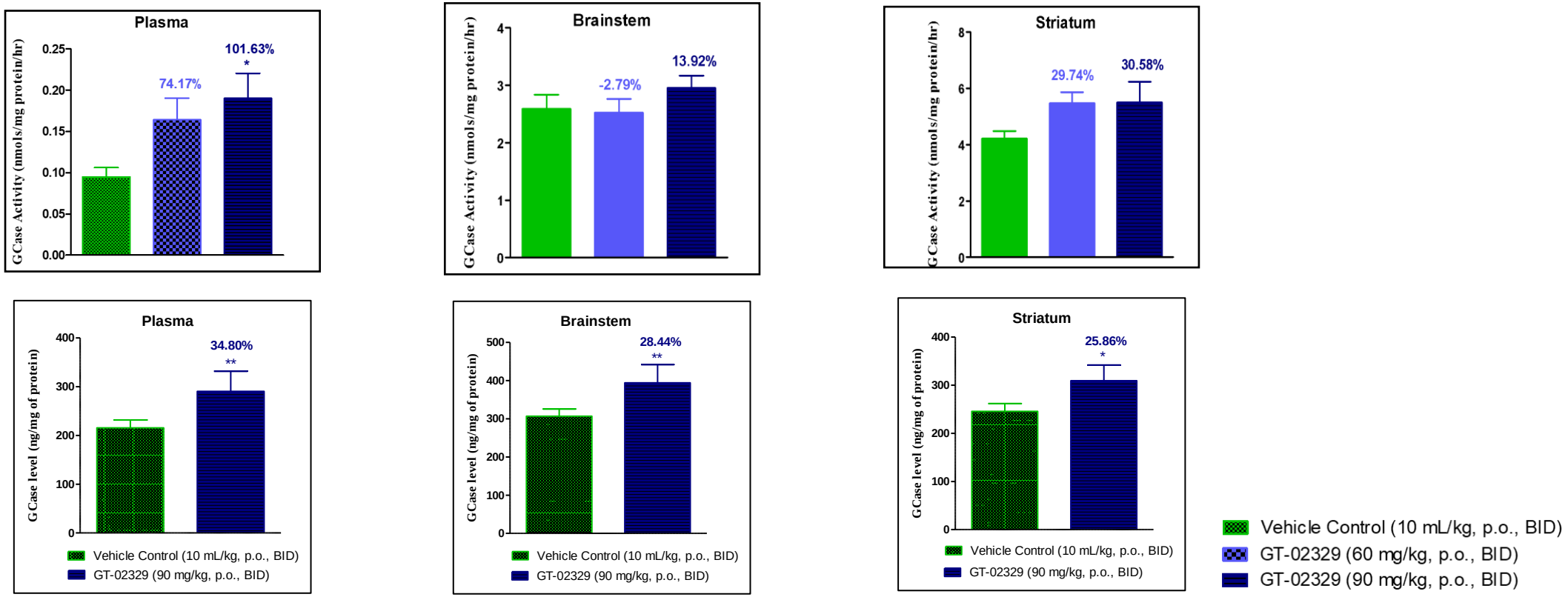




A rat primary culture of mesencephalic neurons was stablished. On day 6, indicated compounds were applied and after 24 hour, CBE (400 µM) was added to the culture medium for 48 hours. On day 8, the culture was fixed and stained with tyrosine hydroxylase (TH), a marker for dopaminergic neurons. Neuronal survival, neurite network and lysosomal pathology parameters were evaluated.

GT-02329
In vivo PoC in Wild-Type C57BL/6 mice

Oral administration of GT-02329 significantly enhances WT GCase activity and protein levels

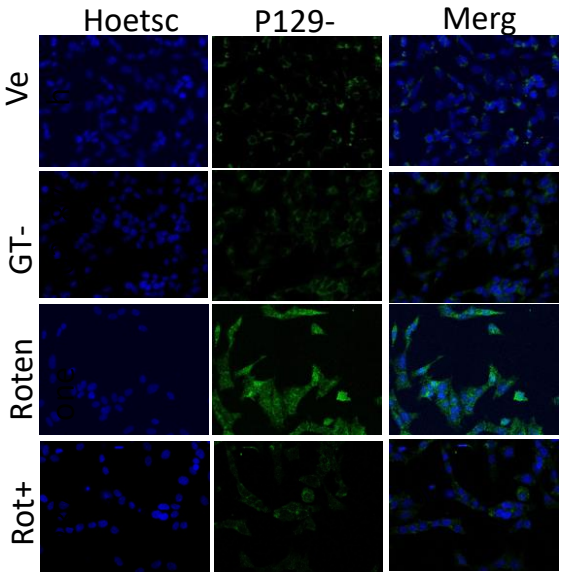


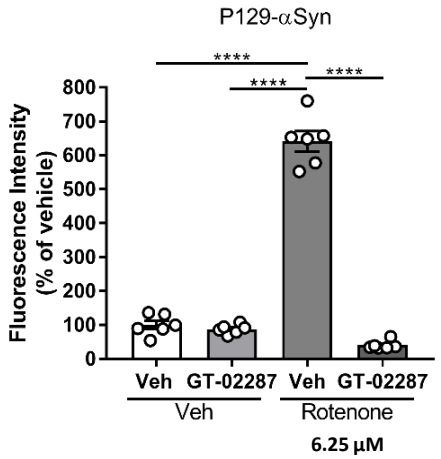
Statistical analysis was performed using One way ANOVA followed by Dunnett's post-hoc test with GraphPad Prism software version 5.0.; All the values are expressed as Mean ± SEM. *p<0.05 and **p<0.01 Vs Vehicle control. GT-02329 was administered orally, twice a day, for 12 days. Samples were collected 1 hr after the last administration. N=10 per group.

GT-02287
PoC in rotenone-induced models of PD

In vitro SH-SY5Y model

GT-02287 reduces α-synuclein accumulation induced by rotenone





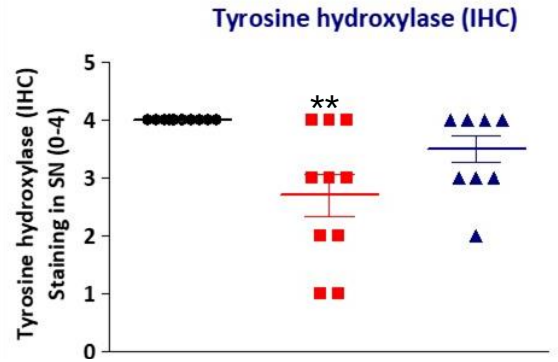
2 days GT -02287 6.25 µM followed by 2 days GT-02287 + rotenone 62.5 nM

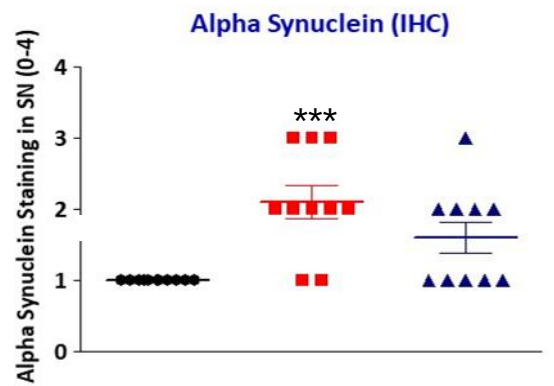
Rotenone is widely used to induced a PD-like pathology in culture cells; Rotenone treatment increased α-syn in total cell lysates suggesting that it reduces SH-SY5Y DAergic neuron viability by promoting α-syn accumulation.

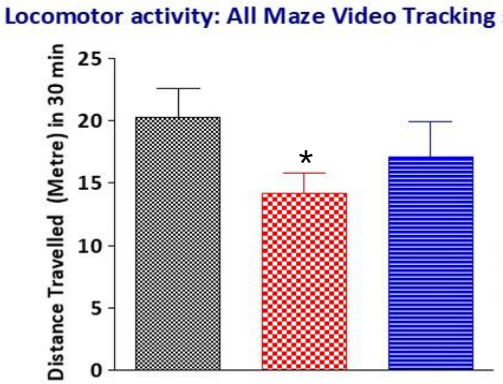
In vivo rat model

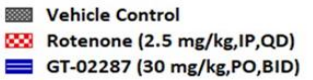
GT-02287 showed a tendency to:

- **Increase TH (dopamine synthesis biomarker)**
- **Decrease alpha synuclein**
- **Improve locomotor activity vs. vehicle treated rats**









The rotenone model of PD reproduces many features of the human disease, including oxidative stress and apoptosis, loss of tyrosine hydroxlase, proteasomal dysfunction, increase of pSer129-α-synuclein, reduced mitochondrial protein import as well behavioral and motor deficits.

Data is shown as Mean ± S.E.M.(n=10) Significant difference as compared Rotenone Vs Vehicle control: *p < 0.05 **p < 0.01 ; *** p < 0.001. GT-02287 was administered orally at 30 mg/kg, twice a day, for 7 days.