Brain-penetrant structurally targeted allosteric regulators for glucocerebrosidase (GCase) show promising pharmacological activity in models of neurodegenerative diseases

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Introduction

Mutations in the *GBA1* gene, encoding the lysosomal enzyme GCase, represent the most common genetic risk factor for Parkinson's disease (PD). Impaired GCase function has earned attention not only due to its association with α-synuclein pathology in GBA-associated PD patients, but also in sporadic PD.

Although less investigated, decreased GCase levels and activity is also associated with the pathophysiology of Alzheimer's disease (AD). Importantly, overexpression of GCase was shown to promote lysosomal degradation of α-synuclein and Amyloid-Beta (Aβ) toxic forms, thus contributing to amelioration of symptoms in PD and AD, respectively. Enhancing the activity of mutant and dysfunctional wild-type GCase may represent a therapeutic strategy for the treatment of neurodegenerative diseases.

β-glucocerebrosidase

Gain Therapeutics has applied the innovative proprietary drug discovery platform, Site-directed Enzyme Enhancement Therapy (SEE-Tx™), to the development of small-molecule structurally targeted allosteric regulators (STARˢ) that stabilize dysfunctional GCase enzyme avoiding its degradation whilst facilitating its maturation and trafficking to the lysosomes.

Through SEE-Tx™, Gain Therapeutics has identified druggable allosteric sites and STARˢ that show a dose-dependent binding to the recombinant WT GCase protein. Importantly, STARˢ are brain penetrant and orally bioavailable, and correct dysfunctional Gcase activity thereby restoring its normal function, with subsequent improvement of lysosomal function and health.

Methods

CBE-induced Parkinson's Disease mouse model

Treatment

Male C57BL/6 mice (8–10-week-old), n=10,
with a treatment duration of 9 days

Group	Treatment	Dose, Route of administration, Dosage volumen
G1	Vehicle Control	Vehicle 1 (Saline, 10 mL/Kg, I.P.) + Vehicle 2 (Compound formulation, 10 mL/Kg, P.O.,BID)
G2	CBE + Vehicle	100 mg/kg, I.P.,QD + Vehicle 2 (Compound formulation, 10 mL/Kg, P.O.,BID)
G3	CBE + GT-02287	100 mg/kg, I.P.,QD + 60 mg/kg, P.O.,BID

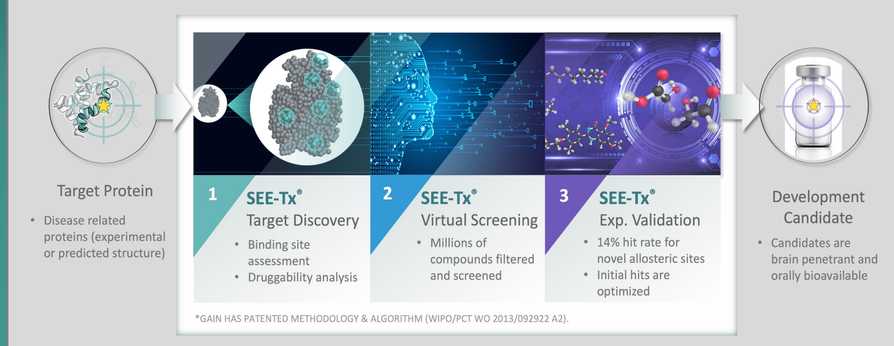
Behavioral test

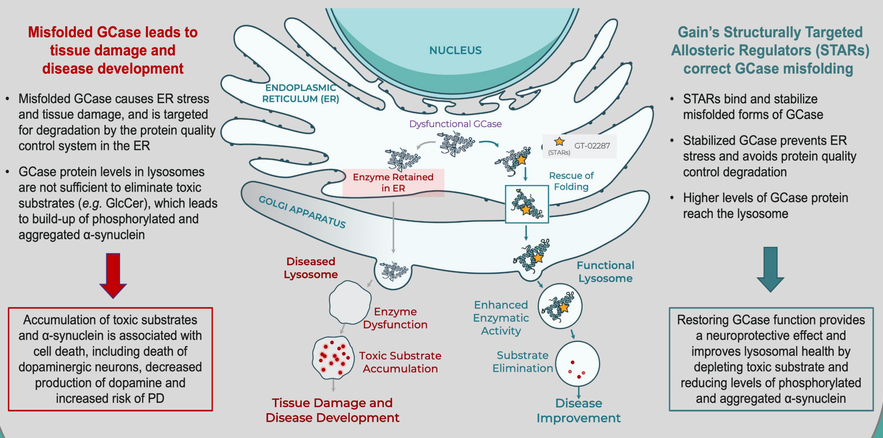
Mice were trained on Day-10 to run on rotarod at 5 rpm for 60 s rotarod. Each mouse was placed on the rod for a session of three trails/day, with a 10-min interval between each trial. On day-11, the speed of rotarod rotation increased at a constant rate (from 4 to 40 rpm) over 300 s. The latency to fall was calculated in s.

Immunostaining: P-alpha synuclein, Aggregated alpha-synuclein, Iba-1 & TH

The staining was visualized by reaction with diaminobenzidine, counterstained with Hematoxylin, and analyzed in a blind-manner under the light microscope. The following score was used: *1= Normal expression 2= Mildly enhanced expression 3= Moderately enhanced expression.4= Markedly enhanced expression*.

Technology
SEE-Tx™ Drug Discovery Platform





Aβ 1-42-induced Alzheimer's Disease neuronal model

Pre-incubation of STAR compound

On day 7 of culture, rat primary cortical neurons were pre-treated with GT STAR compound for several days.

Aβ 1-42 injury

Aβ1-42 peptide was dissolved in the defined culture medium, at an initial concentration of 20 µM. This solution was gently agitated for several days at 37°C in the dark.

Aβ1-42 preparation was added to a final concentration of 15 µM.

Immunostaining: MAP2 & AT100

24 hours after injury, cells were fixed and incubated with:

- antibody anti-microtubule-associated-protein 2 (MAP -2)
- antibody anti-phosphorylated Tau (Ser212/Thr214 (AT100))

Automatic computer analysis

The following endpoints were automatically assessed:

- Analysis of neuron survival (MAP-2 staining, number of neurons)
- Analysis of tau hyperphosphorylation on AT100 (overlap between AT100 and MAP2 staining)
- Analysis of neurite network (MAP-2 staining, total neurite length in µm)

Results

GT-02287 decreases CBE-induced neurotoxicity and motor deficit in a PD mouse model

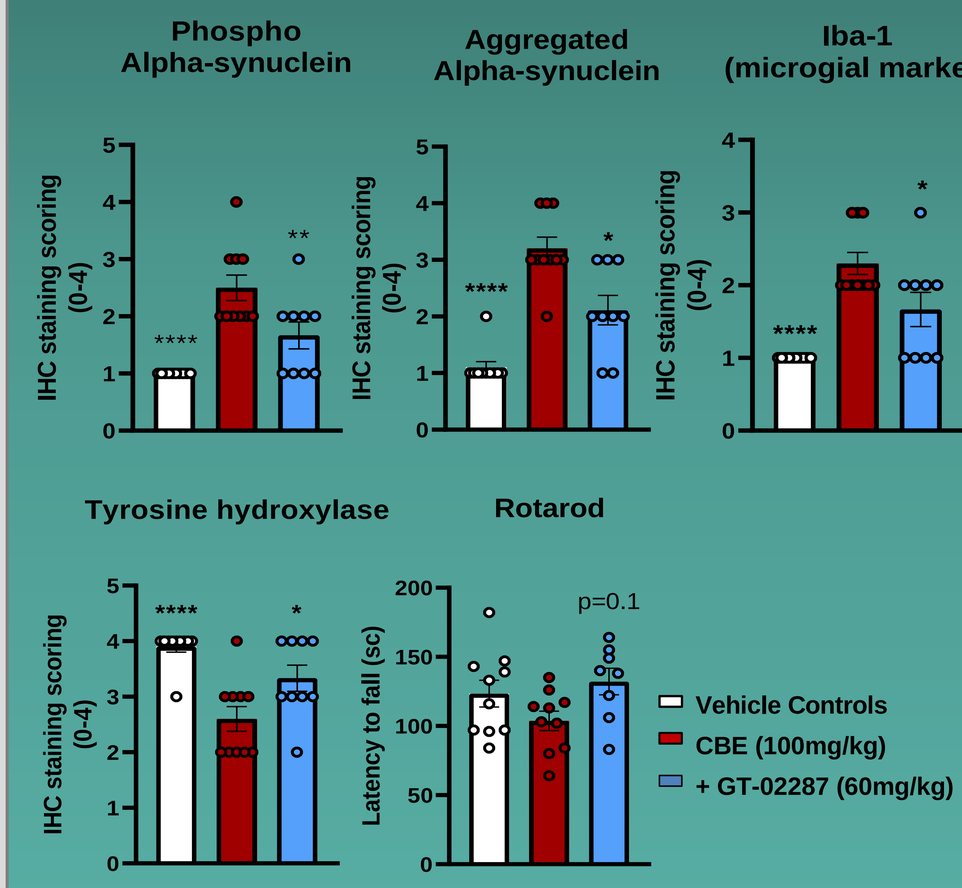


Figure 1. Representative graphs showing immunoexpression of phospho alpha-synuclein, aggregated alpha-synuclein, Iba-1 and tyrosine hydroxylase in the substantia nigra and latency to fall in the rotarod, in vehicle controls (white bar), CBE-treated (red bar) and CBE + GT-02287 (60mg/kg) mice (blue bar). Data is shown as Mean ± S.E.M (n= 8-10), One-way ANOVA followed by Dunnett's Multiple Comparison Test. Significant difference as compared to CBE control. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001

Conclusions

SEE-Tx™ Discovery Platform is:

- ✓ **Highly-specific and cost-effective drug discovery approach**
- ✓ **Efficient in discovering allosteric binding sites that can be targeted for therapeutic benefit to correct protein misfolding diseases, including neurodegenerative diseases.**

GT STARs compounds are shown to significantly:

- ✓ **Decrease toxic forms of alpha-synuclein**
- ✓ **Decrease neuroinflammation**
- ✓ **Decrease hyperphosphorylated tau**
- ✓ **Increase neuronal survival and neurite network**

Improvement of the lysosomal health and function through the stabilization of the enzyme GCase, ameliorates key pathophysiological features in α-synucleinopathies including Parkinson's Disease, as well as in Alzheimer's Disease.

STARs mediated-pharmacological chaperone activity represents a promising therapy that alone or in combination with existent therapies, might prevent or even combat symptoms of neurodegenerative diseases, thus warranting further development towards the clinic.

GT STAR decreases Aβ 1-42-induced neurotoxicity in a *in vitro* neuronal AD model

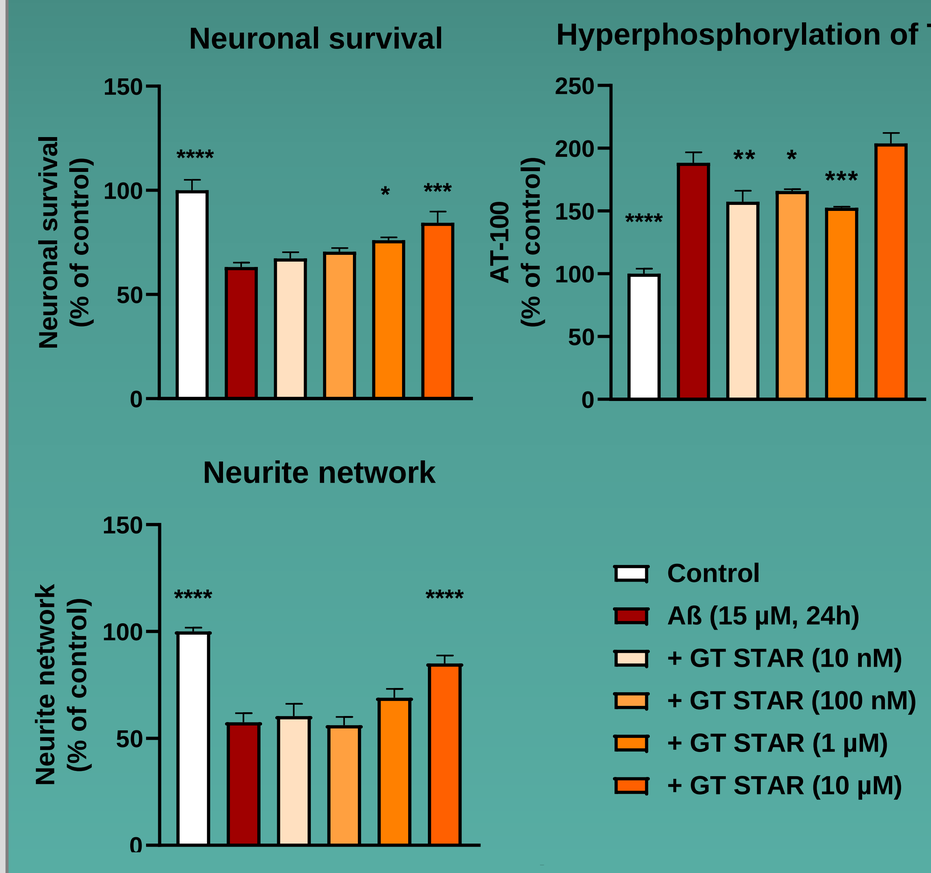


Figure 2. Effects of STAR compound on neuronal survival, on the integrity of the neurite network, and on hyperphosphorylation of tau in a primary culture of cortical neurons injured with Aβ1-42. Data is shown as percentage of control condition as mean +/- SEM (n = 4-6). One-way ANOVA followed by Fisher's LSD test. * Significant difference as compared to Aβ control. *P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001