# Development of structurally targeted allosteric regulators for the treatment of neuronopathic Gaucher disease

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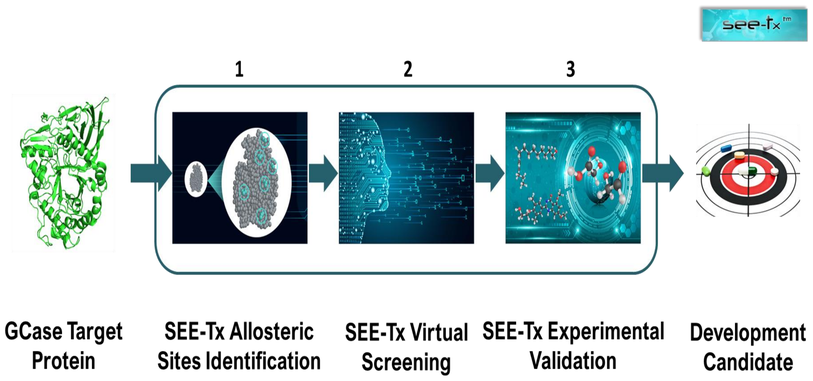
## Abstract

Gaucher disease (GD) is a lysosomal storage disease characterized by deficient activity of the glucocerebrosidase enzyme (GCase) encoded by the *GBA1* gene. This deficiency results in the toxic accumulation of substrates in the liver, spleen, bone marrow, and in severe cases, also in the nervous system. Recent evidence shows that *GBA1* mutant neurons exhibit lysosomal alterations, defective autophagic clearance, accumulation of protein aggregates, and increased vulnerability to cell death. Therefore, targeting these mechanisms could improve and even prevent neurological manifestations. Unfortunately, current approved treatments for GD do not cross the blood-brain barrier thus leaving a huge unmet medical need for the development of novel advanced therapies that can target neuronopathic GD. Gain Therapeutics has applied its innovative proprietary drug discovery platform, Site-directed Enzyme Enhancement Therapy (SEE-Tx™), to the development of small-molecule structurally targeted allosteric regulators (STARˢ) that bind to GCase, stabilize it, and restore its function. The effect of STARˢ treatment on lysosomal function was studied in two different models, namely human GD-iPSC-derived dopaminergic neurons, and a BE(2)-M17 dopaminergic-like neuron model expressing mutant GBA. Our orally bioavailable and brain-penetrant lead STARˢ have shown promising effects in relevant *in vitro* models of GD. STARˢ stabilize misfolded GCase by binding to a site different from its active site ultimately avoiding its degradation while facilitating its maturation and trafficking to the lysosomes. Here we report recent evidence of their beneficial effects on lysosomal function. In two dopaminergic neuronal models, STAR compounds enhance GCase levels, induce the formation of autophagosomes and increase autophagic flux, which in turn leads to a reduction of neurotoxic substrates. Altogether, the *GBA1*-targeted allosteric regulators identified with the proprietary SEE-Tx™ drug discovery platform improve key lysosomal functions thus showing potential as first-in-class therapies for the treatment of neuronopathic GD.

## SEE-Tx™ Discovery Platform




*Gain has patented methodology & algorithm (WIPO/PCT WO 2013/092922 A2).*

## STARˢ Show a Positive Tendency to Increase GCase Protein Levels and Autophagic Flux In A Dopaminergic-like Neuronal Cell Model

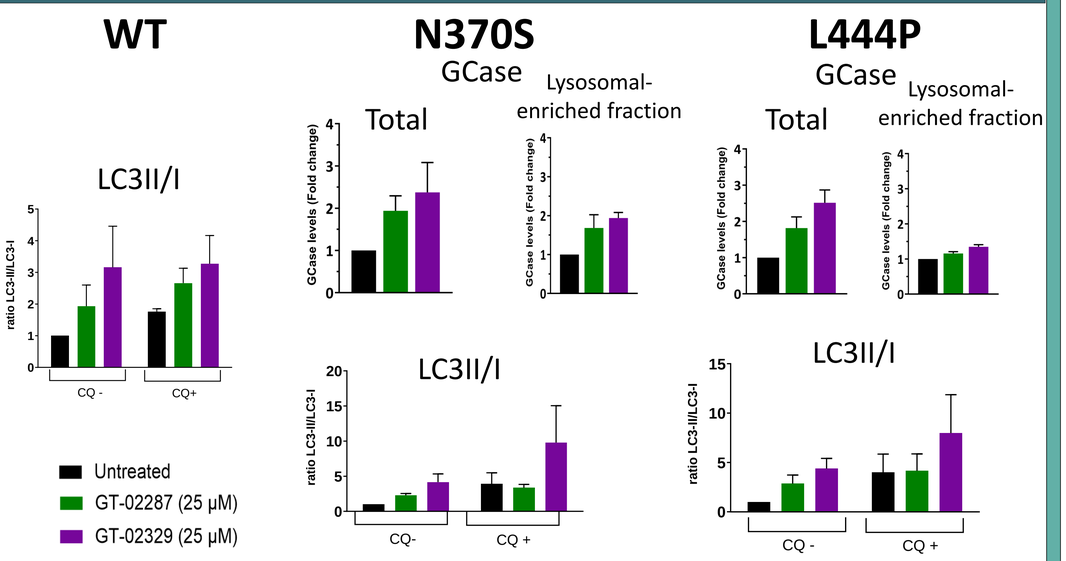



**Fig 1. Effect of STARs on GCase levels and autophagic flux in a dopaminergic-like neuronal cell model.** GCase levels: Dopaminergic neurons BE(2)-M17 carrying either mutant N370S or L444P GBA1 mutations were treated for 4 days with 25 µM of GT compounds. GCase levels were evaluated using western blot in homogenates or lysosomal-enriched fraction and normalized by Ponceau. Results are presented as mean ± s.e.m. values of 4 independent experiments. Autophagic flux: Dopaminergic neurons BE(2)-M17 carrying either wild-type or N370S or L444P GBA1 mutations were treated for 10 days with 25 µM of GT compounds in the presence or absence of 60 µM of the lysosomal inhibitor chloroquine (CQ). The levels of LC3-II and LC3-I were determined by western blot and normalized by Ponceau. Results are presented as mean ± s.e.m. values of 3 independent experiments. Each independent experiment was performed in triplicates. Data was analyzed by 2-way ANOVA.

## STARˢ Increase GCase Protein Levels, Deplete Phosphorylated α-Synuclein and Increase Autophagic Flux In WT and GD iPSC-Derived Dopaminergic Neurons

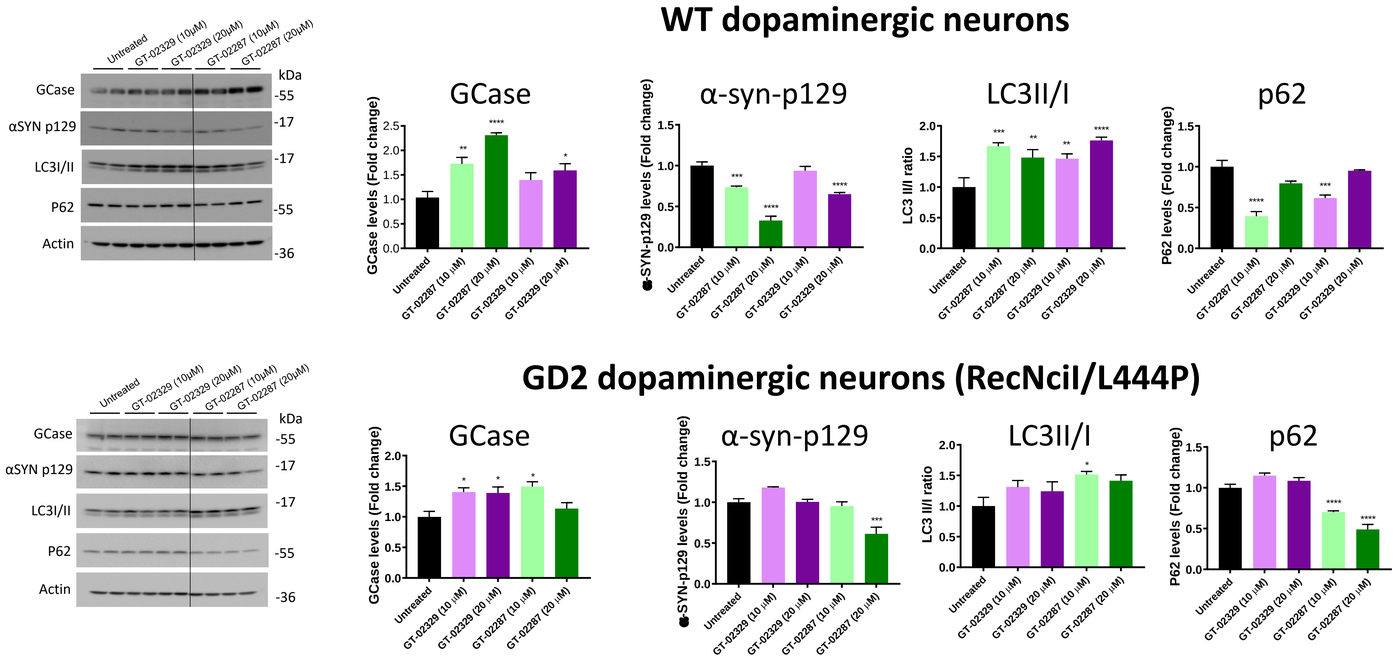



**Fig 2. Effect of STARs on lysosomal function.** WT/WT and Gaucher Disease type II (GD2) iPSc were differentiated to dopaminergic neurons for 60 days as previously described (Awad et al., 2017). Dopaminergic neurons were treated with GT compounds at the indicated concentrations for 48 hours. Levels of GCase, synuclein, LC3-I, LC3-II and p62 were measured by western blot and normalized to actin. Results are presented as mean ± s.e.m values of 4 independent experiments. P values were determined by using one-way ANOVA followed by Bonferroni's multiple comparison test, * p≤0.05, ** p≤0.01, *** p≤0.001, **** p≤0.0001.

## STARˢ Increase GCase Protein Levels And Effectively Reduce the Production of Inflammatory Cytokines in GD iPSC-Derived Macrophages

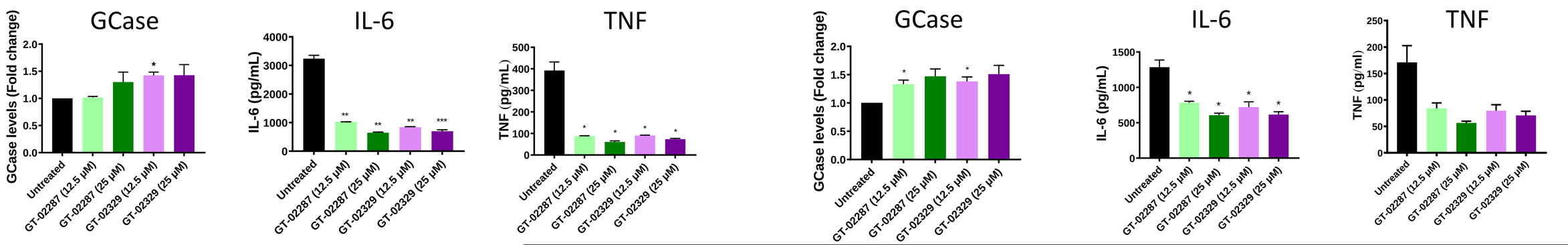



**Fig 3. Effect of STARs on GCase protein levels in GD1 and GD3 macrophages.** iPSc-derived GD monocytes were differentiated to macrophages for 5-7 days (Panicker et al., 2012 and Panicker et al., 2014) After differentiation, the macrophages were treated with the indicated concentrations of each GT chaperone for 3 days. After treatment, cell lysates were prepared and GCase protein levels were analyzed by immunoblotting using antibody to GCase and supernatants were collected and assayed for IL-6 and TNF by ELISA on a Multiplex format on 96-well plates by the University of Maryland Center for Innovative Biomedical Resources (CIBR) Cytokine Core Laboratory. Data was calculated using Luminex's Exponent Software. Results are presented as mean ± s.e.m. values of 3 independent experiments. Unpaired two-tailed student t-test with Welch's correction was done to determine statistical significance (* p<0.05, ** p<0.01, *** p<0.001).

## Conclusions

- SEE-Tx™ is a fast and cost-effective solution that has allowed us to identify structurally targeted allosteric regulators (STARˢ) for GCase enzyme orally bioavailable and brain-penetrant.
- The allosteric GCase STARˢ:
  - Increase total and lysosomal GCase in WT and GD dopaminergic neurons.
  - Lower pathogenic p-α-synuclein129 in WT and GD dopaminergic neurons.
  - Induce the formation of autophagosomes and increase autophagic flux, thus, improving lysosomal function in WT and GD dopaminergic neurons.
  - Increase GCase protein levels in GD macrophages.
  - Effectively reduce the production of inflammatory cytokines by GD macrophages.