Structurally targeted allosteric regulators show promising therapeutic effect in Gaucher Disease cortical neurons

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Abstract

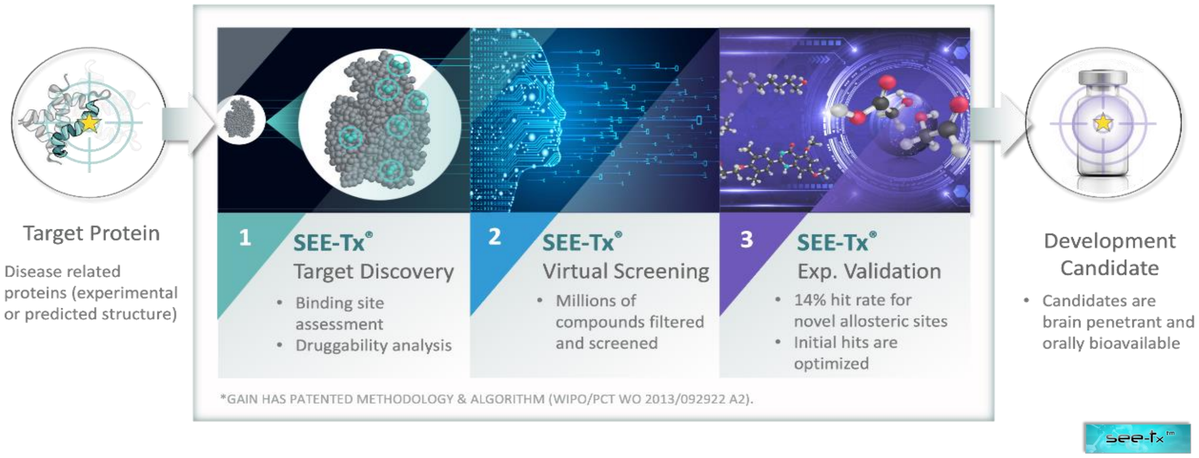
Objective: Biosynthesis and subsequent degradation of glycosphingolipids is a tightly regulated process, and failure of an enzyme to participate in the metabolism results in storage of the enzyme's substrate, giving rise to a lysosomal storage disease. Gaucher disease (GD) is a glycosphingolipid disorder caused by a defect in the catabolic activity of glucocerebrosidase (GCase), causing progressive accumulation of its substrates, glucosylceramide and glucosylsphingosine, predominantly in the lysosome. In severe cases accumulation of these substrates occurs also in the central nervous system and here treatment proves challenging due to restricted access of therapeutics through the blood-brain barrier. For this reason, a high unmet medical need exists for the development of novel advanced therapies that can target GD neurological symptoms.

Methods: 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, stabilizes it and restore its function. To predict the potential effect of STARs treatment for neuronopathic GD, lead compounds were tested in a relevant *in vitro* model based on human WT- and GD-iPSC-derived cortical neurons.

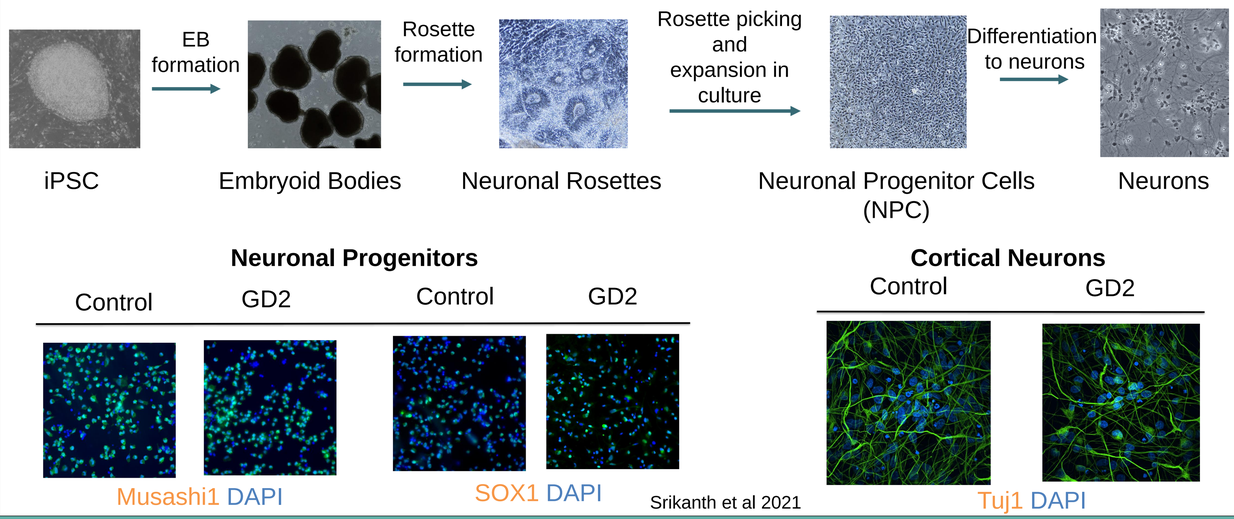
Results: Our orally bioavailable and brain-penetrant lead STARs have shown promising effects in a relevant neuronal model of GD. By binding to an allosteric site on GCase, STARs can stabilize the protein thus avoiding its degradation and facilitating its maturation and trafficking to the lysosomes. In human WT and GD-iPSC-derived cortical neurons STARs show statistically significant increase of GCase protein levels and activity as well as co-localization with lysosomes. Most importantly, our lead compounds decrease toxic substrate accumulation.

Conclusion: Altogether, this data supports the application of allosteric regulators targeting GCase as potential first-in-class therapies for the treatment of GD.

SEE-Tx™ Platform Technology

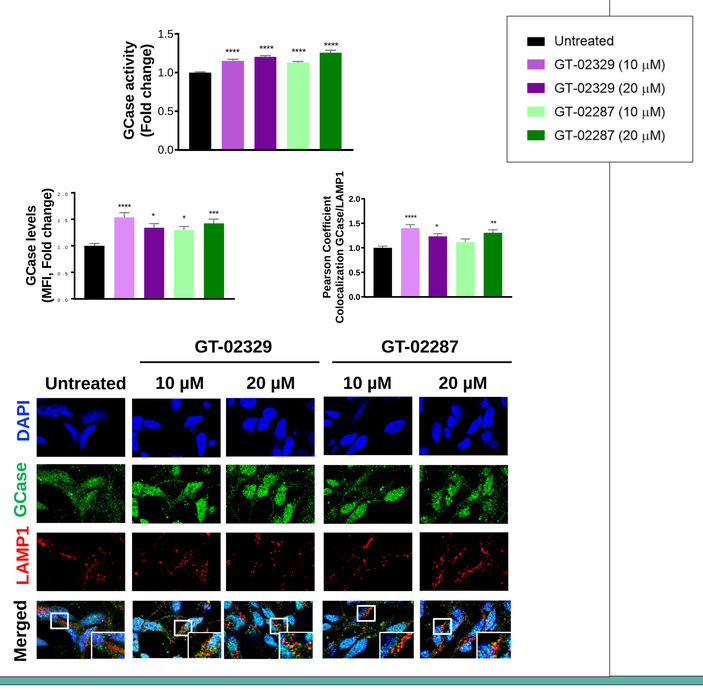


Differentiation of WT and GD iPSc to Cortical Neurons

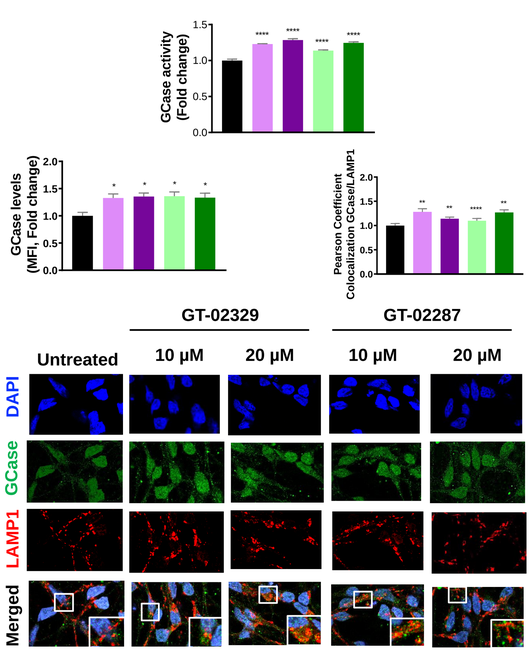


WT Cortical Neurons

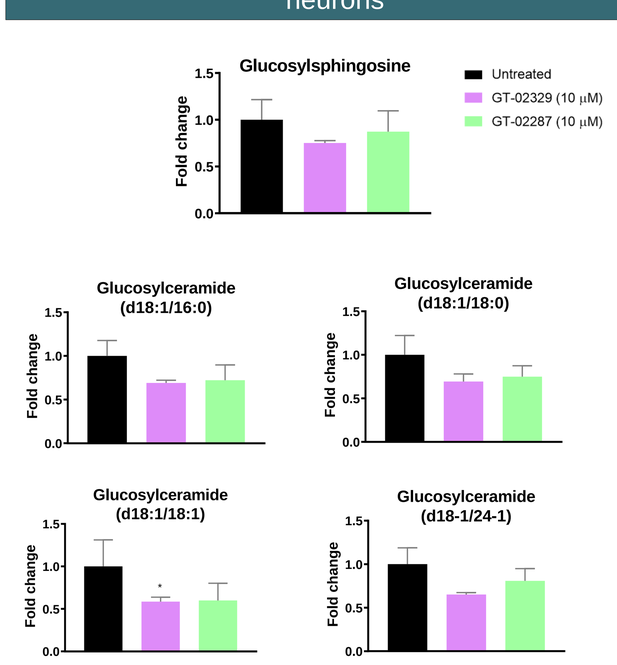
STARs increase GCase activity as well as total and lysosomal GCase levels in WT and GD type III cortical neurons



Gaucher Type III (L444P/L444P) Cortical Neurons

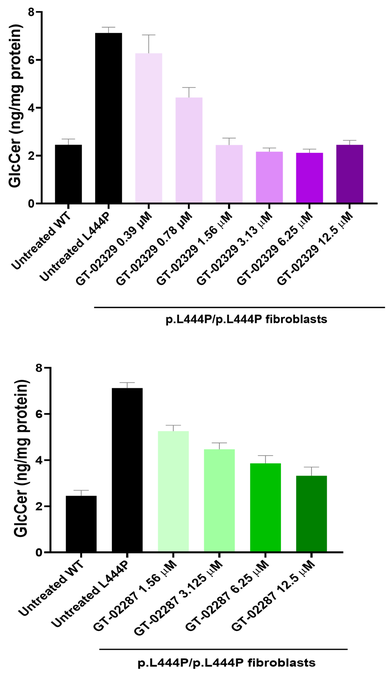


STARs decrease GlcCer and GlcSph in GD cortical neurons



Gaucher Type II (L444P/L444P) Patient-Derived Fibroblasts

STARs decrease GlcCer in GD type II fibroblasts



WT and Gaucher Disease type III (GD3) iPSc were differentiated to cortical neurons as previously described (Srikanth et al., 2021). Cortical neurons were treated with GT compounds at the indicated concentrations for 48 hours. GCase activity was measured using the 4-methylumbelliferyl-beta-D-glucopyranoside substrate. GCase MFI levels and colocalization of GCase/LAMP1 were determined from confocal images analysis using an A1 Nikon confocal laser-scanning microscope. Briefly, the cultures were stained with antibodies to GCase, the lysosomal marker LAMP1, and nuclei were stained with DAPI. Results are presented as mean ± SEM values of 3 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. Glucosylsphingosine and glucosylceramide were quantified by UPLC-MRM/MS. Data were collected from three independent experiments. Results are presented as mean ± SEM. P values were determined by using unpaired, one tailed t-test, * p≤0.05.

p.L444P/p.L444P Gaucher type II patient-fibroblasts were treated with GT-02287 or GT-02329 at the indicated doses in triplicates. Untreated wild-type and p.L444P/p.L444P fibroblasts were also included. After a 10-day treatment, cells was harvested, and glucosylceramide was analyzed by UPLC tandem mass spectrometry by Pronexus. Results are presented as mean ± SEM values.

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:
  - Significantly increase GCase levels and activity in WT and GD cortical neurons.
  - Increase lysosomal GCase levels in WT and GD cortical neurons.
  - Effectively deplete toxic substrate levels in GD cortical neurons and patient-derived Gaucher fibroblasts.