Preclinical development of brain-penetrant structurally targeted allosteric regulators for the treatment of neuronopathic Gaucher disease

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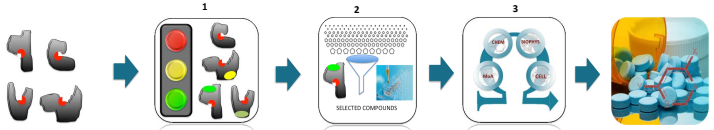
Abstract

Gaucher disease (GD) is a multisystemic lysosomal storage disorder arising from a deficiency of glucocerebrosidase (GCase). Current treatments with enzyme replacement therapy and substrate reduction therapy do not address specific manifestations, particularly neurological, bone and lung symptoms due to their inability to properly reach these tissues. 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 can allosterically bind and stabilize target mutant enzymes thus avoiding their degradation and recovering their enzymatic activity. Here, we report the most recent advancements in the development of lead STAR compounds, which have shown promising effects in different models of neuronopathic Gaucher disease. Indeed, they enhance both enzymatic activity in a dose-dependent manner and substrate depletion in neuronopathic GD patient-derived fibroblasts as well as in a model of dopaminergic neurons. These compounds are orally bioavailable and distribute into multiple tissues, including the most relevant for the disease, i.e., brain and liver. Given these positive features, one of the most advanced molecules is currently being evaluated in a GD3 mouse model with the goal of advancing it toward clinical development

SEE-Tx™ Discovery Platform



Disease-related proteins with orthosteric binding sites. Potential for structurally targeted allosteric regulators

SEE-Tx™ technology identifies allosteric binding sites and assesses their druggability, enabling selection of most promising candidate targets. 50% of protein structures present allosteric sites with good druggability

Multi-million compound libraries are pre-filtered computationally and tested experimentally, providing a list of active compounds. Average hit rate: 10%

Initial hits are evolved in our optimization engine, which considers multiple parameters and generates a non-competitive PC as drug candidate. Orally available and BBB penetrant

Development Candidate

STARs Enhance GBA Activity And Substrate Depletion In Fibroblasts

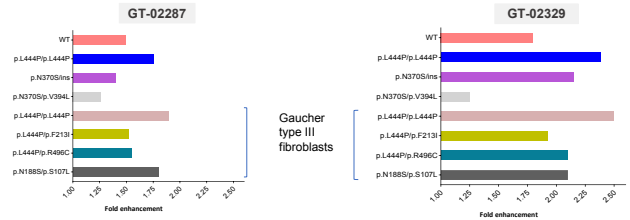


Fig 1. Enzyme Enhancement in a panel of patient derived fibroblasts.

Gaucher patient-fibroblasts or WT fibroblasts were treated with GT-compounds at 12.5 µM. After 4-day treatment, GCase activity was assessed using 4-MU-β-D-glucopyranoside. After a 4-day treatment, GCase activity was assessed using the 4-MU-β-D-glucopyranoside substrate. The assay reaction 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 were incubated at 37ºC for 1h and the reaction was stopped by the addition of 200 µL of glycine buffer (pH 10.7) to each well. Liberated 4-methylumbelliferone was measured (excitation 340 nm, emission 460 nm). Fold increase compared with non-treated cells was calculated.

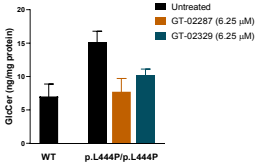
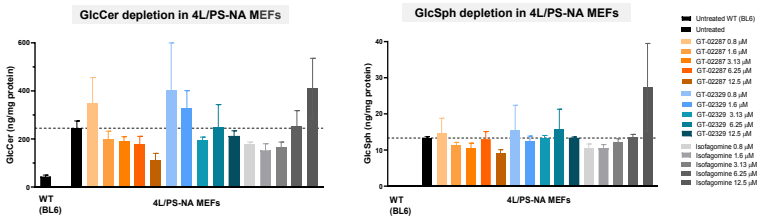


Fig 2. Depletion assay in patient-Gaucher fibroblasts.

p.L444P/p.L444P Gaucher patient-fibroblasts were treated with GT-02287 at 6.25 µM. After a 4-day treatment, cells were harvested and glucosylceramide was analysed by ultra high performance liquid chromatography tandem mass spectrometry by Pronexus.

STARs Reduce GCase Substrates In Gaucher Mice Model Fibroblasts



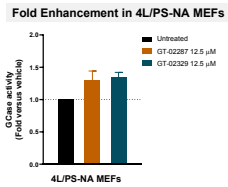


Fig 3. Enzyme Enhancement and substrate depletion in a mice cellular model.

MEFs isolated from the 4L/PS-NA mice were treated with the compounds. After two days, cells were re-treated with fresh medium containing compounds. Untreated BL6 MEFs served as a healthy control. After two additional days, cells were harvested and glucosylceramide and glucosylsphingosine were analysed by UPLCMs-Ms. Fold increase compared with untreated cells is also shown.

STARs Activity In A Dopaminergic-like Neuronal Cell Model

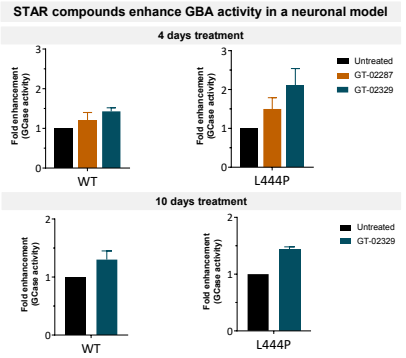


Fig 4. Enzyme enhancement in a neuronal cell model.

Dopaminergic neurons BE(2)-M17 carrying either wild-type or mutant L444P GBA1 mutations were treated for 4 and 10 days with 25 µM of GT compounds. GCase activity was measured with 4-methylumbelliferyl-β-D-glucopyranoside.

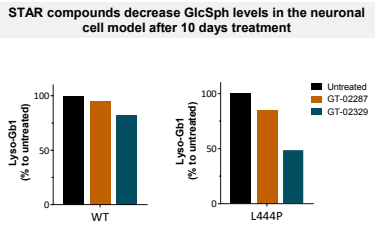


Fig 5. Substrate depletion assay in a cellular neuronal model.

Deaccumulation Assays: Detection of Lyso-Gb1 (Glucosylsphingosine) by LC-MS/MS in BE(2)-M17 cells bearing wild-type and L444P GCase after 10 days treatment with GT-02329 and GT-02287. GBA activity was also assessed using 4-MU-β-D-glucopyranoside substrate. Fold increase compared with untreated cells was as well calculated.

STARs Are Brain Penetrant And Orally Bioavailable

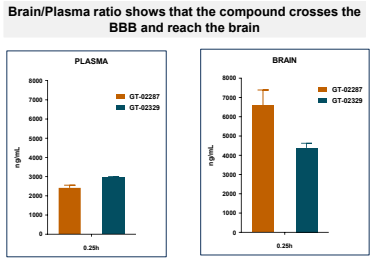


Fig 6. Neuro PK assay: (at 0.25hrs)

Plasma and brain levels were calculated following a single IV administration (C57BL/6, 3 mice, 10 mg/kg GT compound).

A level of 2500 ng/ml corresponds approx. to 5 µM

GT-02287 and GT-02329 exhibited 36% and 35% oral bioavailability respectively

STARs Show A Good PK Profile

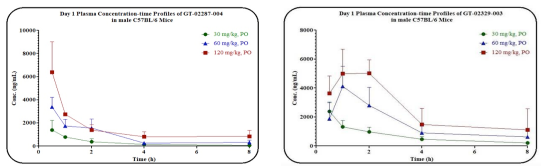


Fig 7. Pharmacokinetics data of GT-02287 and GT-02329

Data produced in male C57BL/6 mice following a single and once daily oral administration for 7 days (Dose: 30, 60 and 120 mg/kg/day)

Conclusions

- Applying its proprietary SEE-Tx™ platform to the Glucocerebrosidase protein, Gain Therapeutics has identified hit series with high efficiency which have been developed into highly promising Lead series.
- Identified Gain's structure-targeted allosteric regulators (STARs) enhance GBA enzymatic activity in several cell types, including human fibroblasts, mice fibroblasts and dopaminergic-like neuronal cells.
- STAR compounds induce an increase of GBA activity in wild type and patient derived fibroblasts, particularly in neuropathic Gaucher patients derived. As well as a significant reduction of the toxic substrate Glucosylceramide accumulated in neuropathic L444P fibroblasts.
- In a neuronal model, STAR compounds show GBA activity enhancement and Glucosylsphingosine reduction.
- Gain's compounds are active in a dose dependent manner in the Gaucher in vitro model 4L/PS-NA mice fibroblasts. They enhance GBA activity and modulate depletion of the toxic substrates Glucosylceramide and Glucosylsphingosine.
- STAR Gain's compounds are brain penetrant and oral bioavailable.

For more information about the mechanism of action of STARs, please refer to poster LB-40 (Insights into the mechanism of action of structurally targeted allosteric regulators for the treatment of Gaucher disease).