Insights into the mechanism of action of structurally targeted allosteric regulators for the treatment of Gaucher disease

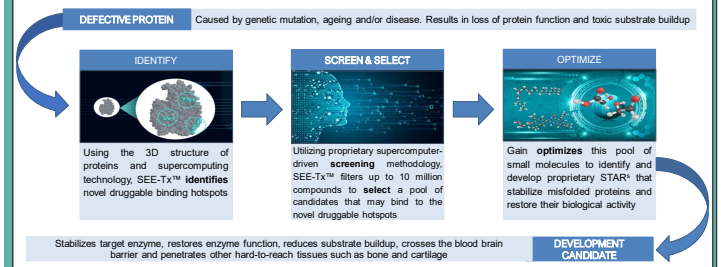
N. Pérez-Carmona[1], A. M. García-Collazo[1], E. Cubero[1], T.J. Bergmann[2], A. Ruano[1], A. Delgado[1], S. Morales[1], M. Molinari[2,3] and X. Barril[1]

[1]Gain Therapeutics, Barcelona (Spain) sites [2]Institute for Research in Biomedicine-USI, Bellinzona (Switzerland) [3]Ecole Polytechnique Fédérale de Lausanne, School of Life Sciences, Lausanne (Switzerland).

Abstract

Gaucher disease (GD) is a multisystemic lysosomal storage disorder arising from a deficiency of glucocerebrosidase (GCase) and consequent accumulation of its unprocessed substrates, namely glucosylceramide and glucosylsphingosine. Gain Therapeutics has applied its proprietary computational 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 recent insights into the mechanism of action of lead STAR compounds, which have so far shown promising effects in *in vitro* models of Gaucher disease. Indeed, they stabilize mutant GCase in a non-inhibitory manner and support its proper folding therefore rescuing it from early degradation in the endoplasmic reticulum and allowing its maturation and trafficking to the lysosome. Most importantly, they enhance both enzymatic activity and substrate depletion in patient-derived fibroblasts. All together this data supports and validates the application of SEE-Tx™ as an innovative drug discovery platform for the identification of allosteric regulators for the treatment of Gaucher disease.

SEE-Tx™ Discovery Platform



Identification of a new allosteric binding sites

- The published human GCase 3D structure obtained by X-ray crystallography and refined to 1.95 Å resolution was used (PDB ID: 2v3f).
- Molecular dynamics simulations of the protein in organic-aqueous solvent mixtures (**MDmix**) reveal a druggable cavity.
- MDmix was also used to identify key interaction sites (binding hot spots), which were used as pharmacophoric restraints to guide docking, and to explore the conformational flexibility of the binding site.

3D structure of the GCase monomer

Hit ID by Virtual Screening

- A virtual collection of >6 million commercially-available compounds were evaluated computationally with the docking program **rDock** using the standard scoring function, pharmacophoric restraints and a high-throughput protocol.
- Best scoring compounds were visually inspected and 102 were selected based on the plausibility of the binding mode and chemical diversity considerations.
- Screening by DSF afforded 13 hits (**13% hit rate**). Hit validation was based on SAR-by-catalogue, which provided 7 additional active compounds (24% hit rate).

STARˢ Increase Lysosomal GCase

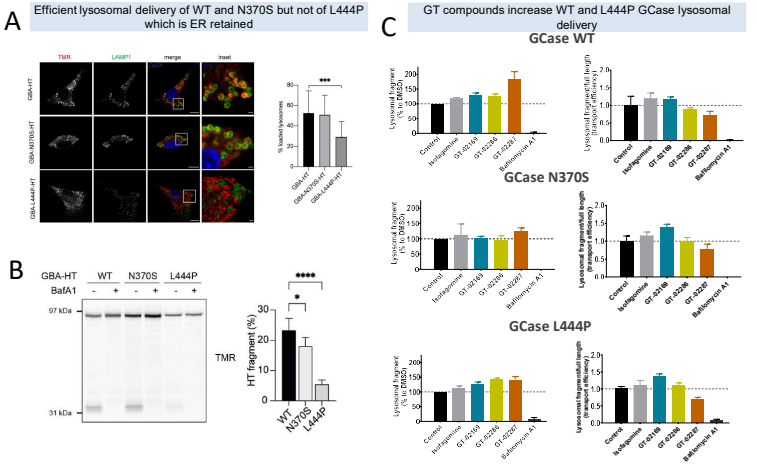


Fig 3. Halo-Tag assay for measurement of trafficking in living cells. WT, N370S and L444P-GCase variants characterization by Confocal-laser scanning microscopy and HT-assay. A) Representative confocal IF images of MEF cells expressing GCase-HT variants for 48 h and exposed 17 h to 50 nM BafA1 and a fluorescent Halo ligand (TMR). Quantification of the percentage of lysosomes filled with TMR. Images analyzed with LysoQuant (Morone et al., 2020). Statistical analysis: One-way ANOVA followed by Dunnett's multiple comparison test, ***p<0.001. **B) and C)** HEK293 were transfected with either GCase WT, GCase N370S and GCase L444P with HaloTag. 30 hours later, TMR and compounds (25 µM) or 50 nM BafA1 were added for 17 hours. Samples were collected, subjected to SDS-page and the fluorescent signal was analyzed. Once the protein reaches the lysosome, the tag is cleaved off GCase but is resistant to lysosomal hydrolases enabling the detection of a 31 kDa fluorescent fragment that corresponds to the protein that reached the lysosomes.

STARˢ Specifically Bind and Stabilize GCase

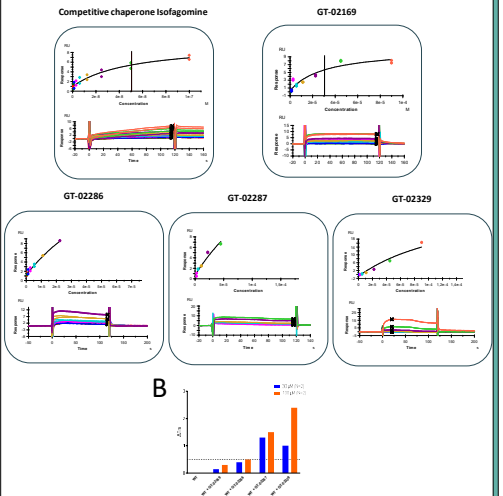


Fig 1. STARˢ bind and stabilize β-galactosidase. A) Direct binding studies with STARˢ were performed by Surface Plasmon Resonance (SPR). Evaluation of the SPR results indicates that STARˢ show dose-response binding to the immobilized GCase protein (Cerezyme®) monitored at neutral pH 7.4. **B)** In order to investigate if binding of STARˢ to GCase has an effect on stability, a thermal shift assay with GCase in absence and presence of STARˢ were performed by the differential scanning fluorimetry method (nanoDSF, a tryptophan fluorescence thermal shift assay). Results indicate that STARˢ stabilize GCase in a dose-response manner.

STARˢ Do Not Inhibit GCase

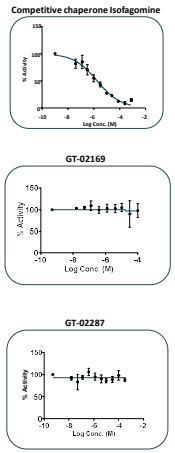


Fig 2. STARˢ do not inhibit GCase. Cell lysates from WT fibroblasts are incubated with compounds at several doses and the samples are assayed for GCase activity using 4-methylumbelliferyl β-D-glucopyranoside. Data is expressed as mean ± SD (N=2).

STARˢ Are Active in the Low Micromolar Range in WT and Gaucher Patient-Derived Fibroblasts

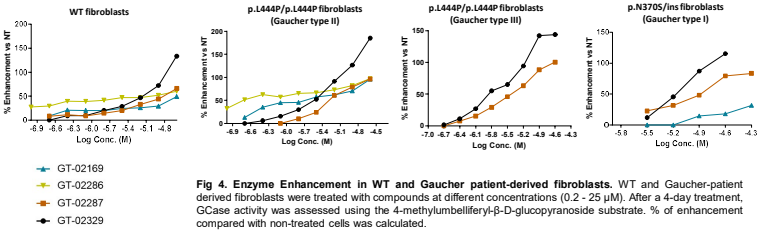


Fig 4. Enzyme Enhancement in WT and Gaucher patient-derived fibroblasts. WT and Gaucher-patient derived fibroblasts were treated with compounds at different concentrations (0.2 - 25 µM). After a 4-day treatment, GCase activity was assessed using the 4-methylumbelliferyl-β-D-glucopyranoside substrate. % of enhancement compared with non-treated cells was calculated.

Conclusions

- SEE-Tx™ is a fast and cost-effective solution that has allowed us to identify structurally targeted allosteric regulators (STARˢ) for GCase enzyme.
- The allosteric GCase STARˢ:
  - Specifically bind and stabilize the target protein GCase and do not inhibit the enzyme.
  - Increase lysosomal GCase.
  - Enhance GCase activity in WT and a panel of Gaucher patient-derived fibroblasts bearing prevalent mutations.
  - STARˢ are drug-like, orally bioavailable, brain penetrant compounds and have a positive in vitro and in vivo acute toxicity profile offering excellent therapeutics opportunities.
- For the characterization of STARˢ in additional cell models as well as its evaluation of the in vivo PK profile, please refer to poster LB-17 (Preclinical development of brain-penetrant structurally targeted allosteric regulators for the treatment of neuronopathic Gaucher disease).