Brain Penetrant Structurally Targeted Allosteric Regulators for Treating GLB1-Related Disorders

M. Bellotto[1,*], R. Maj[1], A. M. García-Collazo[2], A. Ruano[2], A. Delgado[2], S. Morales[2], E. Cubero[2], N. Pérez[2], X. Barril[2]

[1] Gain Therapeutics SA, Lugano, Switzerland; [2] Gain Therapeutics Switzerland, Barcelona Branch, Spain

* Contact: mbellotto@gaintherapeutics.com

ABSTRACT

Loss of activity of lysosomal β-galactosidase (GLB1) causes a group of disorders, that include neuronopathic GM1 gangliosidosis and non-neuronopathic Morquio B disease, due to the accumulation of the enzyme substrates. There are currently no approved therapies which reverse the effect, and there is an unmet need for developing treatments that can ameliorate a patient's condition and especially the bone, cartilage and neurological manifestations. Gain Therapeutics is focused on the development of small-molecule drugs for the treatment of lysosomal diseases such as GM1 gangliosidosis. To achieve that, Gain Therapeutics relies on the proprietary technology SEE-Tx (Site-directed Enzyme Enhancement Therapy) to develop structurally targeted allosteric regulators (STAR), small-molecules that stabilize the defective enzymes avoiding their degradation and recovering their enzymatic activity. Among them, allosteric non-inhibitory regulators offer advantages when compared with competitive, normally sugar-like, pharmacological chaperones by not competing with the natural substrate. SEE-Tx has allowed Gain to identify a druggable allosteric site in the β-galactosidase protein and small molecule binders that stabilize the enzyme and enhance its activity. We report the identification of four lead compounds which bind to the target enzyme in a non-inhibitory manner and enhance the enzyme activity in *in-vitro* cell line assays. GT leads have shown to be orally bioavailable, non-toxic up to 400 mg/kg in mice and distribute into multiple tissues, including the most relevant for the disease as brain, cartilage, and bone. These compounds are promising new candidates for the treatment of GM1-gangliosidosis and Morquio B patients holding β-galactosidase mutations sensitive to pharmacological chaperoning and are currently being evaluated with the goal of advancing toward clinical development.

DISCOVERY

Identification of a new allosteric binding sites

- The published human GLB1 3D structure obtained by X-ray crystallography and refined to 1.8 Å resolution was used (PDB ID: 3thc).
- 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 GLB1 monomer

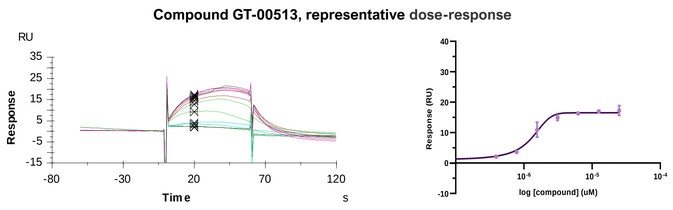
Hit ID by Virtual Screening

- 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 80 were selected based on the plausibility of the binding mode and chemical diversity considerations.
- Screening by DSF afforded 3 hits (**4% hit rate**). Hit validation was based on SAR-by-catalogue, which provided 6 additional active compounds (14% hit rate).

STAR MOLECULES

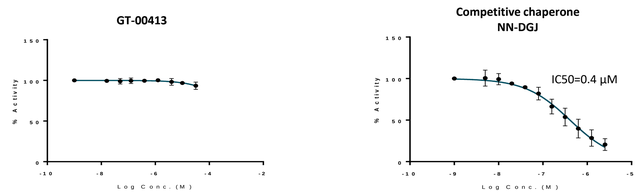
1) Allosteric stabilization of the purified enzyme

STAR lead compounds bind GLB1



The SPR technique detects changes in the refractive index on the surface of a sensor (due to mass changes). These changes have been used to measure the biomolecular interaction between the purified GLB1 protein and GT lead compounds.
Data shows clear evidence of direct binding. Similar affinity obtained among compounds with a range of Kd 1-4 µM. Data are expressed as mean ± SD (*n*=2).

STAR lead compounds do not inhibit GLB1



Cell lysates from WT fibroblasts are incubated with GT-00413 or the competitive chaperone NN-DGJ at several doses and the samples are assayed for GLB1 activity using resorufin beta D-galactopyranoside. GT-00413 and the other lead compounds have no inhibitory activity at concentrations as high as 33.3 µM whereas NN-DGJ has an IC50 of 0.4 µM. Data are expressed as mean ± SD (*n*=2).

2) Enhancement of enzymatic activity and substrate reduction in canine fibroblasts

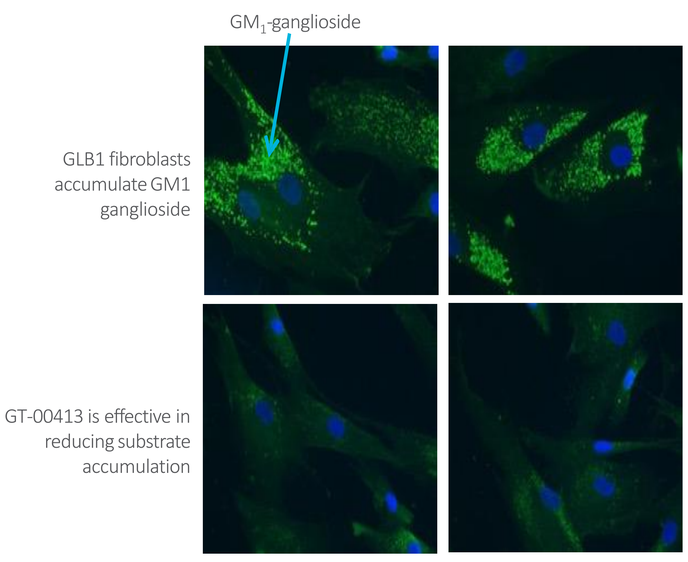
STAR lead compounds enhance GLB1 enzymatic activity

Enzyme enhancement in p.R60H/p.R60H canine fibroblasts (Equivalent to human R59H)

Treatment	Fold
Untreated	1
GT-00413 (25 µM)	1.24 (*n*=11)
GT-00493 (25 µM)	1.26 (*n*=11)
GT-00513 (25 µM)	1.22 (*n*=10)
GT-00546 (25 µM)	1.25 (*n*=7)

GM1 gangliosidosis fibroblasts were treated with the indicated compounds. After a 4 day treatment, GLB1 activity was assessed using resorufin beta-D-galactopyranoside. Fold increase (treated cells/untreated cells) of GLB1 activity is presented as mean ± SD. The number of independent experiments is shown in brackets.

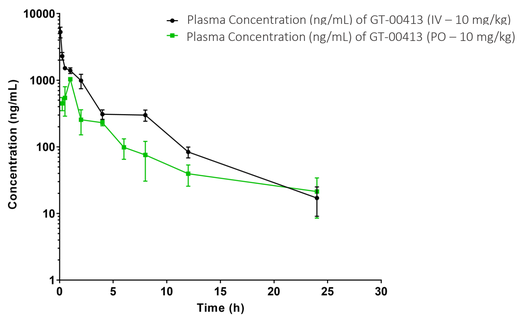
GT-00413 reduces GM1-ganglioside accumulation



GM1 gangliosidosis canine fibroblasts (p.R60H/p.R60H) were loaded with GM1 ganglioside for 2 days followed by culture in the presence of GT-00413 at 25 µM for 4 subsequent days. The cells were fixed, permeabilized and stained to detect GM1 ganglioside. Nuclei were counterstained with DAPI.

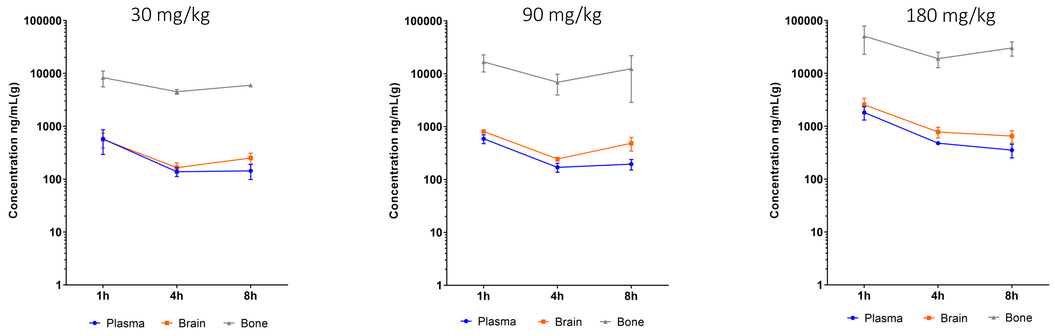
3) In vivo: Oral Bioavailability, PK and Toxicity profiles

STAR lead compounds are orally bioavailable



Plasma pharmacokinetics of GT-00413 following a single intravenous and oral administration to male C57BL/6 mice. Data are expressed as mean ± SD (*n*=3).
GT-00413 oral bioavailability F = 38%.

STAR lead compounds are brain and bone penetrant



Plasma pharmacokinetics and tissue (brain and left femur bone) distribution of GT-00413 following a single oral (180 mg/kg) and 7 day repeated oral (30 and 90 mg/kg) dose administration in male C57BL/6 mice.
GT-00413 was well tolerated after 7 days treatment without showing clinical signs. Comparable plasma exposure was observed at 30 and 90 mg/kg po and accumulation was not observed after 7 days treatment. Brain concentrations were quantifiable up to 8 hours showing good brain and excellent bone exposure. Single oral dose of 180 mg/kg was quantifiable in plasma, brain and bone up to 8 hours. Dose linearity was observed after 90 and 180 mg/kg. Data are expressed as mean ± SD (*n*=3).

STAR lead compounds have a positive acute toxicity profile

GT-00413, GT-00493, GT-00513 and GT-00546 were administered once as suspensions (0.5% w/v Na CMC in water) to male C57BL/6 mice by oral (gavage) route at doses 50, 150 and 400 mg/kg. No abnormal clinical signs and mortality were found.

Tolerable dose was **≥ 400 mg/kg**

CONCLUSIONS

Gain Therapeutics' SEE-Tx platform provides a very efficient way of discovering structure-targeted allosteric regulators (STAR). Its application to the β-galactosidase protein resulted in a **4% hit rate**. The hits represent several chemical series that were further validated and tested through our screening cascade. Four STAR lead compounds have been further characterised for their potential to treat GLB1-related disorders as they:

- **Bind directly to the enzyme in a non-competitive way**
- **Enhance enzymatic activity in fibroblasts**
- **Reduce toxic substrate accumulation in fibroblasts**
- **Penetrate brain and bone tissues**
- **Have a positive acute toxicity profile and are not mutagenic (mini Ames test)**
- **Have low off-target activity**
- **Have half-life suitable for once daily use**
- **Are orally bioavailable**

Contact

Manolo Bellotto, PhD
General Manager & President

T. +41 91 921 1524
www.gaintherapeutics.com

Download