# Identification of novel allosteric β-galactosidase regulators that prevent GM1 ganglioside accumulation

Ana Ruano[1], Maria Pons-Vizcarra[1], Ana Trapero[1], Aida Delgado[1], Xavier Barril[1], Manolo Bellotto[1], Joanne Taylor[1], Elena Cubero[1], Natàlia Pérez-Carmona[1] Ana María García-Collazo[1]

*[1]Gain Therapeutics, Lugano (Switzerland) and Barcelona (Spain)*

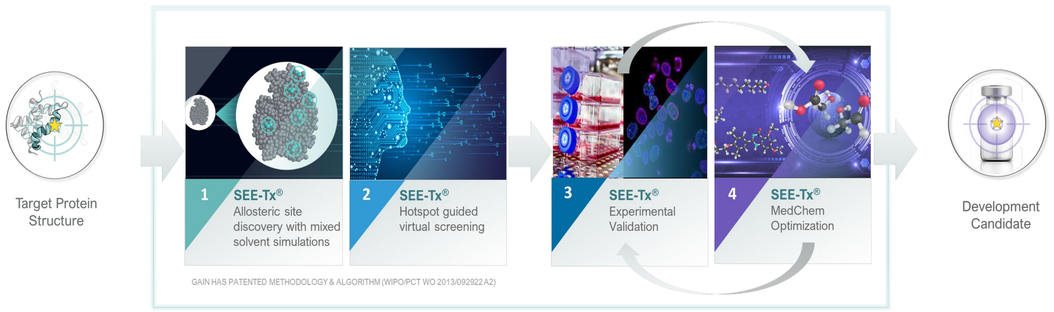
## Abstract

Loss of lysosomal β-galactosidase activity causes a group of disorders that include neuronopathic GM1 gangliosidosis and non-neuronopathic Morquio B disease, due to the accumulation of the enzyme's substrates. There are currently no approved therapies that can prevent or reverse disease progression, thus creating a high unmet medical need for patients with these conditions. Using our proprietary drug discovery platform SEE-Tx® (Site-directed Enzyme Enhancement Therapy), we have identified allosteric binding sites and small hit molecules that were developed into structurally targeted allosteric regulators (STARs) for the treatment of lysosomal diseases such as GM1 gangliosidosis. STARs have been shown to correct protein folding of mutated enzymes, thus avoiding their degradation and restoring their enzymatic activity.

To determine the mechanism of action and activity of the putative β-galactosidase STARs, we have developed a cell-based fluorescent imaging screening assay to assess alleviation of exogenous GM1 ganglioside accumulation in a GM1 gangliosidosis cell model. By applying this technology, we have identified a novel series of potent compounds that were confirmed to bind β-galactosidase enzyme and deplete GM1 ganglioside in a dose-dependent manner, which provides an opportunity for the development of new small molecule therapies for GM1 gangliosidosis and Morquio B disease.

## SEE-Tx® Platform Identified STARs for β-Gal enzyme

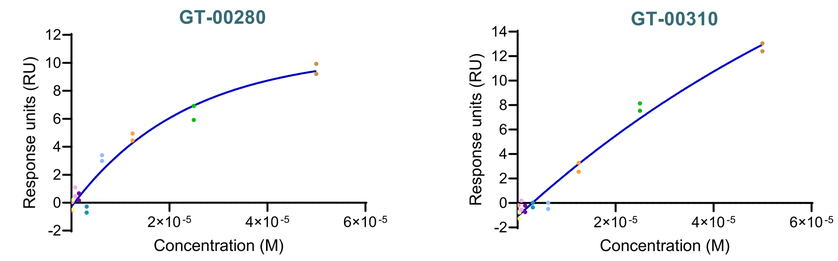
- The SEE-Tx® platform technology, using the 3D structure of the human β-Gal protein (PDB ID: 3THC) and supercomputing technology, discovered an undescribed allosteric binding site and predicted its druggability.
- Applying our proprietary screening methodology, 6 million compounds were filtered to fit the target site. As a result, a set of small molecules that may bind to the novel druggable hotspots was selected.




- Top-scoring hits were confirmed experimentally and yielded one preferred hit series.
- The hit series was the starting point for developing STARs that stabilize misfolded β-Gal proteins and restore their biological activity. Several rounds of design-synthesis-testing assays led to proprietary series of compounds, and the most promising compounds were further characterized in biological assays.

## STARs Bind β-Gal in a Dose-Response Manner

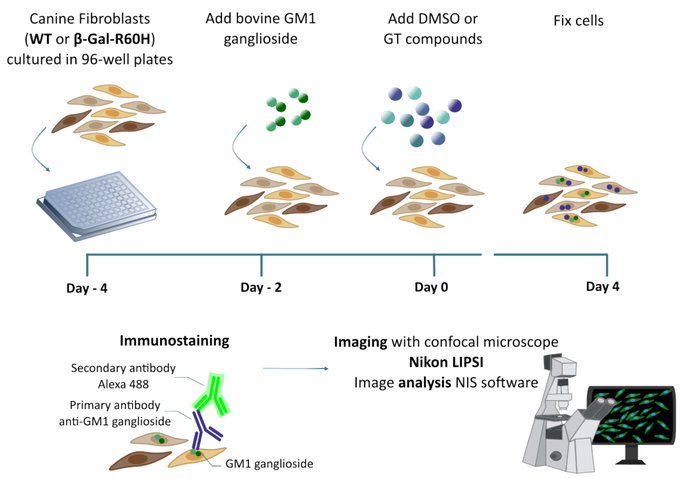
### Direct binding of GT compounds to β-Gal was confirmed using SPR




SPR (surface plasmon resonance) dose-response curves for representative compounds. Compounds were tested at pH 7 in a 2-fold dilution series starting at 50 µM. A protein is immobilized on a sensor chip surface and a sample containing the compound in solution is injected over the surface through a series of flow cells. The SPR technique detects changes in the refractive index on a sensor's surface due to mass variations. These changes are used to measure the biomolecular interaction between recombinant human β-Gal WT protein and STARs.

## STARs Decrease Exogenous GM1 Ganglioside Accumulation In Canine Fibroblasts

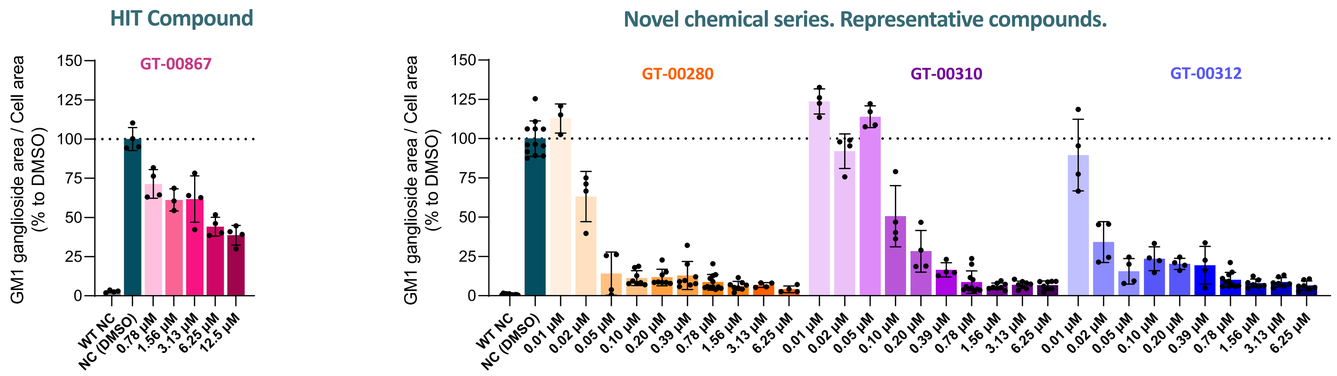
### METHODOLOGY

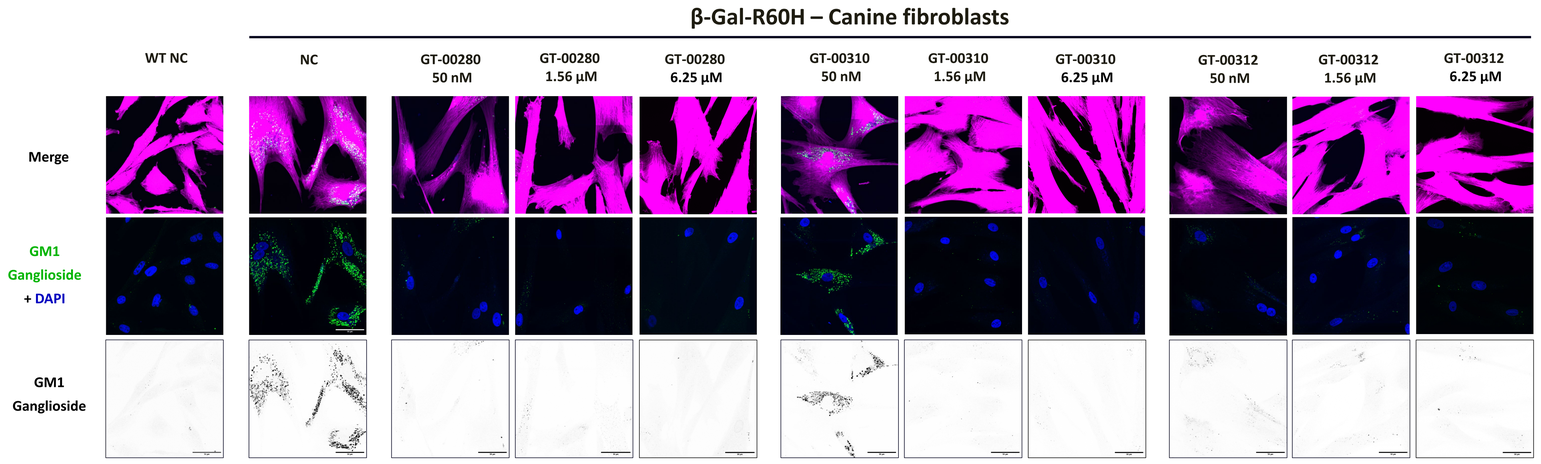



Cell-based fluorescence immunocytochemistry (ICC) screening assay was performed.

- Canine fibroblasts, WT or carrying R60H GLB1 mutation (equivalent to the R59H prevalent mutation in human β-Gal), were seeded in a 96-well plate.
- The fibroblasts were supplemented with exogenous GM1 ganglioside for 2 days followed by incubation of STARs at indicated doses for 4 subsequent days.
- Cells were fixed, permeabilized and stained with anti-GM1 ganglioside antibody (green), CellMask (magenta) to delimit the cell area and nuclei were counterstained with DAPI (blue).
- **Imaging** was performed in a Nikon LIPSI confocal microscope (Advance Digital Microscopy Core Facility, at IRB). 40X objective was used in 3 channels. 25 images/well in each channel were acquired with 10% overlap (5x5). Images presented with 50 µm scale bar.
- **Images analysis** was performed with NIS elements software. % of GM1 ganglioside area/cell area with respect to non-treated cells was determined. Results are presented as mean ± SD.

### Exogenous substrate depletion by GT compounds was confirmed using ICC




## Conclusions

- By applying its SEE-Tx® platform technology, Gain Therapeutics has identified structurally targeted allosteric regulators (STARs) of the β-Galactosidase enzyme.
- Gain Therapeutics has established a medium throughput immunocytochemistry-based assay for the assessment of exogenous substrate reduction.
- A novel chemical series was developed using the ICC assay. Binding of the compounds to the target enzyme has been confirmed.
- The novel allosteric β-Gal STARs series significantly decreases exogenous GM1 ganglioside accumulation in GM1 gangliosidosis fibroblasts in a dose response manner.

GAIN THERAPEUTICS

Contact:
aruano@gaintherapeutics.com