Allosteric regulators improving biodistribution of recombinant laronidase in Mucopolysaccharidosis type 1 (MPS1)

X. Barril,[1,*] E. Cubero,[1] A. Delgado,[1] S. Morales,[1] A. Ruano,[1] N. Pérez,[1] D. Grinberg,[2] L. Vilageliu,[2] M. García-Morant,[2] R. Maj,[3] A. M. García-Collazo[1]

* Contact: xbarril@gaintherapeutics.com

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

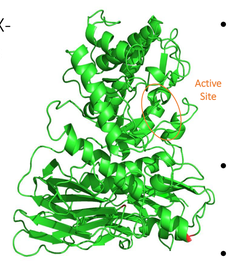
ABSTRACT

Mucopolysaccharidosis type 1 (MPS1) is a lysosomal storage disease also known as Scheie, Hurler-Scheie or Hurler syndrome in relation to its severity. MPS1 is caused by mutations in the alpha-L-iduronidase (IDUA) gene, resulting in enzyme deficiency and accumulation of the IDUA substrates, namely dermatan sulfate (DS) and heparan sulfate (HS). Current treatment is based on enzyme replacement therapy (ERT), where the exogenous IDUA enzyme (laronidase) is administered intravenously once per week. As in other ERTs, the short half-life of the enzyme and the desensitisation caused by its immunogenicity are limiting factors to its efficacy. This results in low bioavailability, particularly in tissues with limited or absent blood circulation such as bone and cartilage, where treatment shows scarce efficacy. Increasing the effective concentration of the enzyme could overall increase the efficacy of the current treatment. ¬¬We have applied the proprietary SEE-Tx technology to discover **structure-targeted allosteric regulators (STAR) of IDUA**: these are **drug-like molecules which can induce conformational stabilization** and increase the thermal stability of normal recombinant enzymes. Combination therapy with pharmacological chaperones and ERT can improve tissue uptake and reduce ERT's immunogenicity by stabilizing the enzyme in its properly folded and active form. **Here we present in vitro and in vivo efficacy of our STAR molecules**. **They protect laronidase from pH-dependent denaturalisation and increase the uptake of the enzyme by MPS1 patient-derived fibroblasts**. When co-administered with laronidase intravenously to WT mice, our STAR molecules **increase its plasma activity levels**, with a peak at 0.5 h and **prolong its enzymatic activity across multiple tissues, including bone and cartilage**. These results open new treatment perspectives in diseases with hard-to-treat organs such as bone and cartilaginous tissues for which an unmet medical need exists.

DISCOVERY

Identification of a new allosteric binding sites

- The published human IDUA 3D structure obtained by X-ray crystallography and refined to 2.3 Å resolution was used (PDB ID: 3w81).
- 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 IDUA monomer

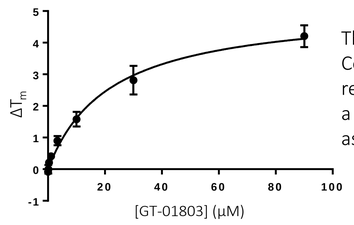
Hit ID by Virtual Screening

- 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 111 were selected based on the plausibility of the binding mode and chemical diversity considerations.
- Screening by DSF afforded 7 hits (6% hit rate). Hit validation was based on SAR-by-catalogue, which provided 14 additional active compounds (19% hit rate), including GT-01803.

STAR MOLECULES

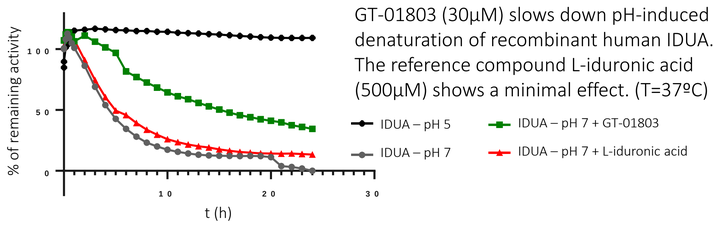
1) Allosteric stabilization of the purified enzyme

GT-01803 increases IDUA thermal stability



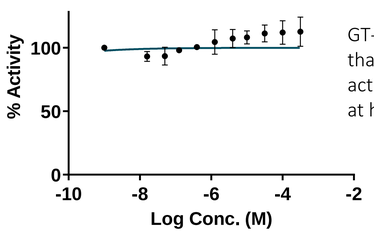
Thermal shift dose-response curve. Compound GT-01803 stabilizes recombinant human IDUA by up to 4ºC on a differential scanning fluorimetry (DSF) assay. K_D = 22μM

GT-01803 prevents IDUA denaturation



GT-01803 (30μM) slows down pH-induced denaturation of recombinant human IDUA. The reference compound L-iduronic acid (500μM) shows a minimal effect. (T=37ºC)

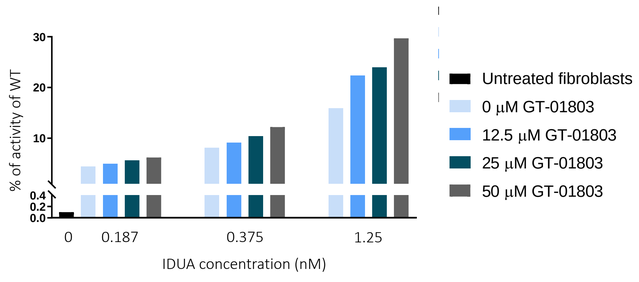
GT-01803 does not inhibit IDUA



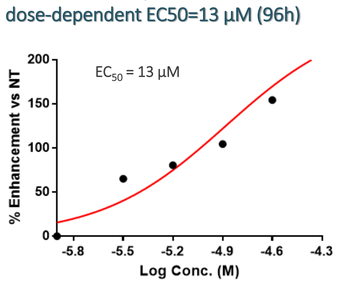
GT-01803 is a silent allosteric modulator that does not affect the intrinsic enzymatic activity of recombinant human IDUA even at high concentrations.

2) Enhanced cellular activity in combination therapy

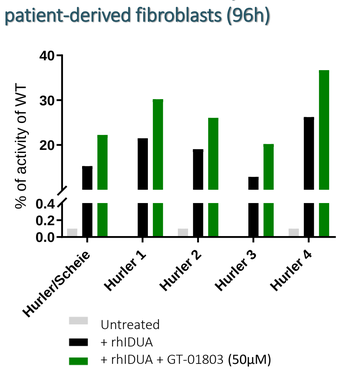
GT-01803 co-administration promotes IDUA stability and cell uptake (96h)



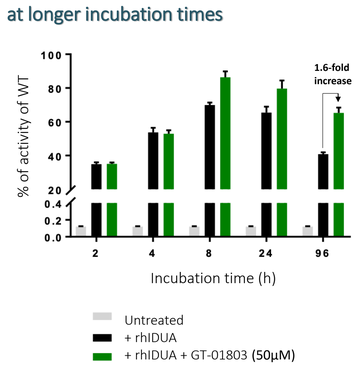
GT-01803 activity enhancement is dose-dependent EC50=13 μM (96h)



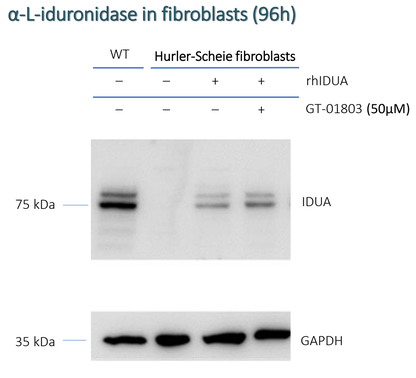
GT-01803 is active across a panel of patient-derived fibroblasts (96h)



GT-01803 beneficial effect increases at longer incubation times



GT-01803 increases the amount of α-L-iduronidase in fibroblasts (96h)

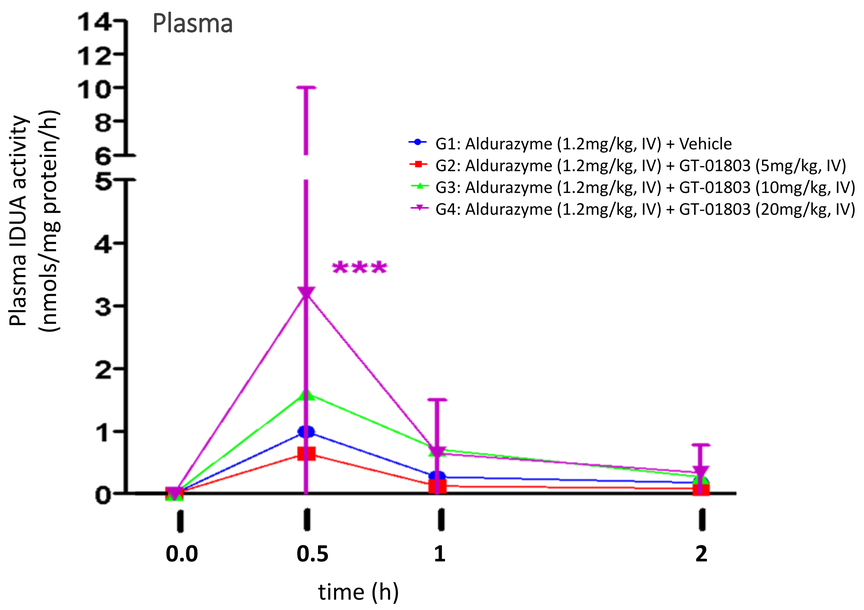


IDUA activity increases in a dose-dependent manner after the addition of rhIDUA to the culture medium in Hurler-Scheie fibroblasts (Basal activity: 0.23) Co-administration with GT-01803 shows a marked increase of IDUA cell activity (vs. single agent) at three different concentrations and in dose-dependent manner. The cell-based EC_{50} (13 μM) is in good agreement with the K_D by DSF (22 μM).

3) In vivo: combination therapy improves PK and tissue activity of Laronidase

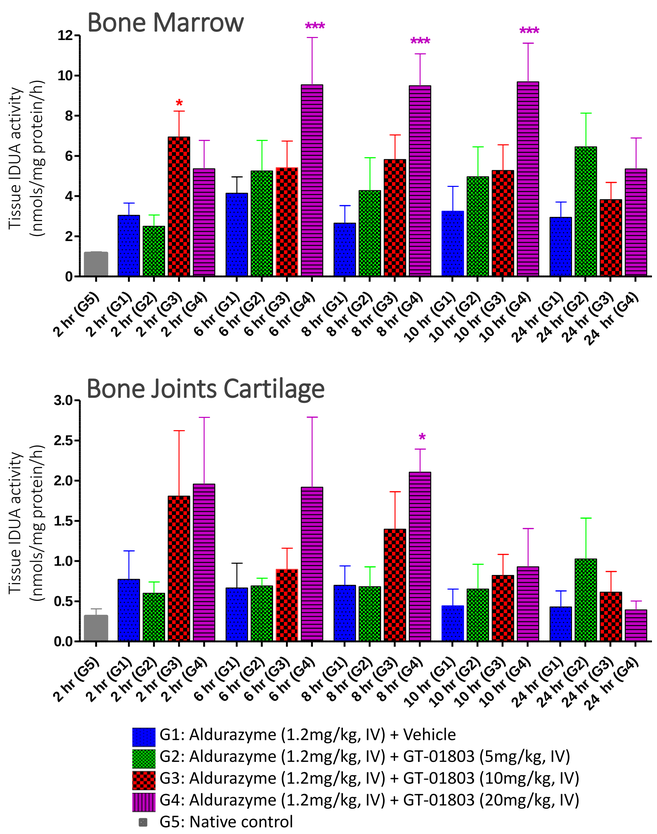
Co-administration of Laronidase with GT-01803 stabilizes the recombinant enzyme, increasing enzymatic activity levels in plasma, liver, bone and cartilage in a dose-dependent manner. Bone and cartilage represent the most burning medical need due to poor ERT uptake. The benefit of combination therapy is particularly noticeable at longer times.

GT-01803 co-administration improves the PK profile of Laronidase



(*** p<0.01)

GT-01803 co-administration increases Laronidase tissue exposure



CONCLUSIONS

- Gain Therapeutics' SEE-Tx platform provides a very efficient way of discovering structure-targeted allosteric regulators (STAR). Its application to the alpha-L-iduronidase (IDUA) protein resulted in a 6% hit rate. The hits represent several chemical series that were further validated in a hit expansion exercise.
- GT-01803, an advanced hit with good development potential, was characterised in biophysical and biochemical assays. It stabilizes the folded protein without affecting its enzymatic activity.
- Cell-based assays demonstrate the potential of GT-01803 as an ERT combination therapy. It increases uptake of rhIDUA protein and its enzymatic activity under a wide range of conditions (rhIDUA concentration and cell-types). Benefits are particularly evident at longer incubation times (≥ 4 days).
- In vivo, GT-01803 improves the PK profile of Laronidase, increasing its plasma levels in a dose-dependent manner. It also increases IDUA enzymatic activity in a panel of tissues, including those that benefit the least from ERT (bone marrow, cartilages). GT-01803 is safe at all doses tested (IV MTD 45 mg/kg).

Contact

Xavier Barril, PhD
Chief Scientific Officer

T. +34 93 403 55 27
www.gaintherapeutics.com

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