GT-02287, A CLINICAL STAGE GLUCOCEREBROSIDASE REGULATOR FOR THE TREATMENT OF PD, EASES ER STRESS AND ENHANCES LYSOSOMAL ENZYME ACTIVITY

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OBJECTIVE

Glucocerebrosidase (GCase) deficiency is linked to pathophysiological features in Parkinson's disease (PD), including heightened endoplasmic reticulum (ER) stress. This dysregulation contributes to the accumulation of aggregated alpha-synuclein, leading to dopaminergic neuron degeneration. This study aims to uncover the mechanism by which GT-02287, Gain Therapeutics' PD drug candidate, targets GCase and impacts the underlying biological processes of PD.

METHODS

Gain Therapeutics applied its proprietary computational drug discovery platform to the identification of the orally bioavailable and brain penetrant GT-02287. GCase function, ER stress and protein quality control were evaluated in patient-derived fibroblasts harboring mutated GCase. A mutated GCase-HaloTag-HEK293 cell-based model was used to measure GCase transport to the lysosome.

RESULTS

MISFOLDED L444P GCASE PROTEIN IS RETAINED IN THE ER, INDUCING ER STRESS

L444P GCase shows enhanced association with ER chaperones

L444P GCase binds to calnexin (CNX) and BiP to greater extent than WT and N370S

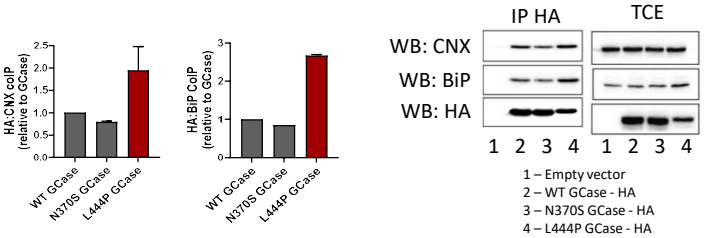

Figure 1. HEK293 cells were transfected with WT, N370S or L444P GCase-HA. After 48h, co-IP with HA and WB was conducted to assess the association of GCase with CNX and BiP. Protein content of total cell extracts (TCE) is also shown.

L444P GCase triggers an unfolded protein response (UPR)

L444P GCase fibroblasts show higher level of the ER stress marker BiP

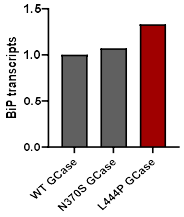

Figure 2. Gaucher patient-fibroblasts (Coriell) bearing N370S (GM00372) or L444P (GM08760) GCase or WT (GM03377) GCase from a healthy individual were cultured and the steady state transcripts of BiP mRNA levels were monitored by qPCR.

GT-02287 ALLEVIATES ER STRESS

GT-02287 mitigates UPR by decreasing BiP levels and other selected UPR markers

GT-02287 inhibits proteasomal degradation of L444P GCase

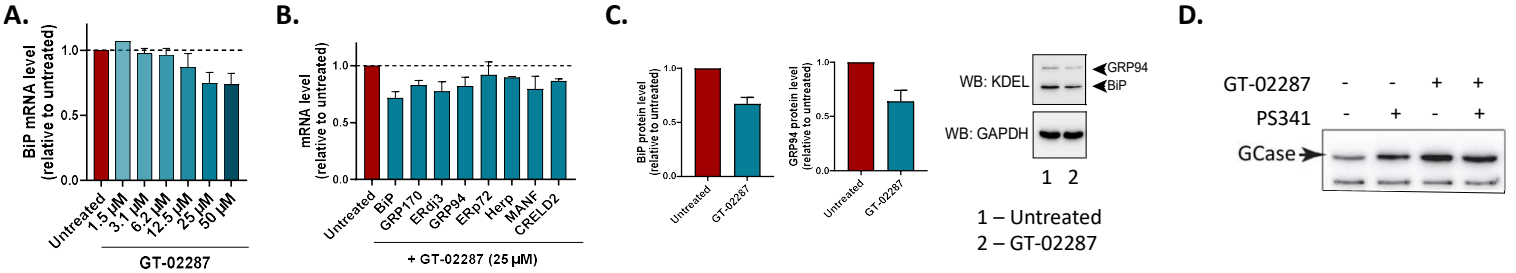

Figure 3. (A,B) L444P fibroblasts (Coriell, GM08760 (A) or GM10915 (B)) were treated with GT-02287 for 4 days (A) or 10 days (B). mRNA levels of selected UPR markers (Bergmann et al., 2018) were evaluated by qPCR. (C) L444P fibroblasts (Coriell, GM08760) were exposed to 25 µM of GT-02287 for 4 days. BiP and GRP94 protein levels were analyzed by WB. (D) Same fibroblasts in C were treated with 25 µM of GT-02287 for 4 days and with the proteasome inhibitor PS341 (10 µM) for 3h. GCase levels were examined via WB.

THIS MECHANISM OF ACTION SUPPORTS THE FINAL OUTCOMES OF GT-02287 TREATMENT BY BOOSTING LYSOSOMAL GCASE ACTIVITY, WHICH REDUCES TOXIC ACCUMULATION OF SUBSTRATE

GT-02287 improves lysosomal GCase transport

GT-02287 increases GCase activity and reduces GlcCer accumulation

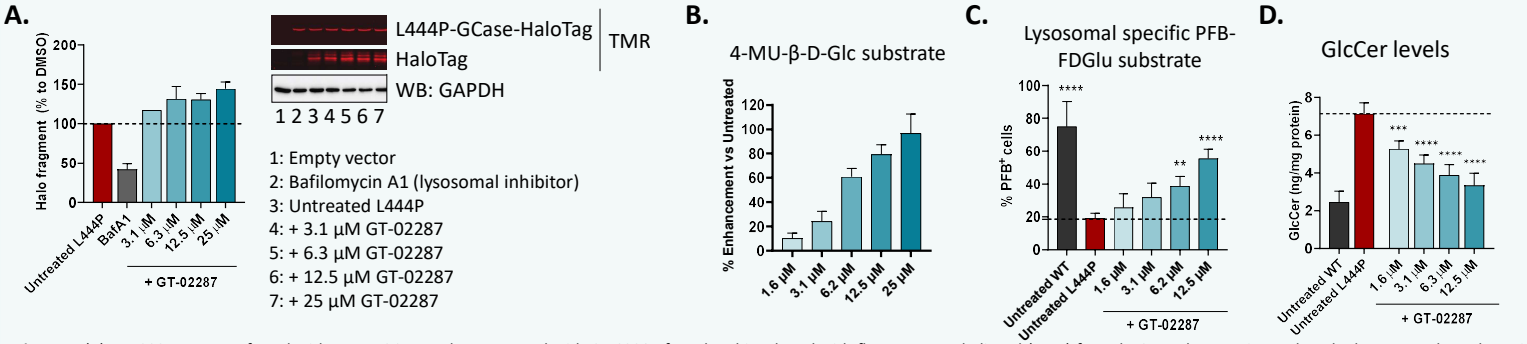

Figure 4. (A) HEK293 were transfected with L444P-GCase-HaloTag, treated with GT-02287 for 4d and incubated with fluorescent Halo ligand (TMR) for 17h. Once the protein reaches the lysosome, the HaloTag is cleaved off GCase but is resistant to lysosomal hydrolases enabling the detection of the fluorescent Halo fragment that corresponds to the protein that reached the lysosomes. After exposure, gel was transferred onto a membrane and immunoblotted against GAPDH. (B, C) L444P patient fibroblasts (Coriell, GM08760) treated with GT-02287 for 4d. GCase activity was assessed using the indicated substrates. (D) Same L444P fibroblasts treated with GT-02287 for 10d. Glucosylceramide was analyzed by UHPLC-MS/MS by Pronexus. **** p≤0.0001, *** p≤0.001, ** p≤0.01 versus Untreated L444P. One-way ANOVA followed by Dunnett's Multiple Comparison Test.

CONCLUSIONS

GT-02287 aids in the correct folding of GCase and prevents it from undergoing protein quality control-mediated ER retention and associated degradation. This enhancement in stability facilitates GCase trafficking into lysosomes, where it can efficiently process its substrate, contributing to lysosomal and cellular health.

GAIN THERAPEUTICS

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