Development of allosteric regulators that bind to α1-antitrypsin and reduce its polymerization

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Introduction

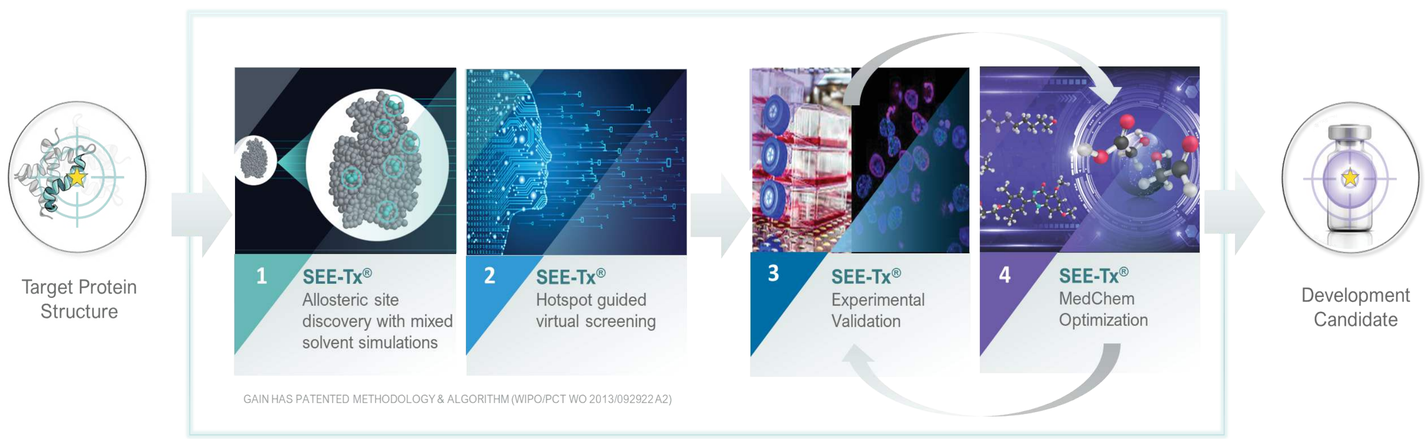
α1-antitrypsin (A1AT) deficiency is caused by a mutation in the gene SERPINA1, the most common variant being the Z allele. This mutation results in misfolding and polymerization of A1AT protein which accumulates in hepatocytes causing liver damage. In addition, polymerized A1AT cannot be secreted, resulting in A1AT not reaching the lungs and insufficient protection of the lungs from neutrophil elastase, leading to early-onset emphysema. There are currently no effective therapies that treat the underlying conditions, creating a high unmet need for a novel and effective therapeutic for these patients.

SEE-Tx™ Technology

Using the SEE-Tx® platform technology, Gain Therapeutics has identified an allosteric binding site in the A1AT protein and predicted its druggability.

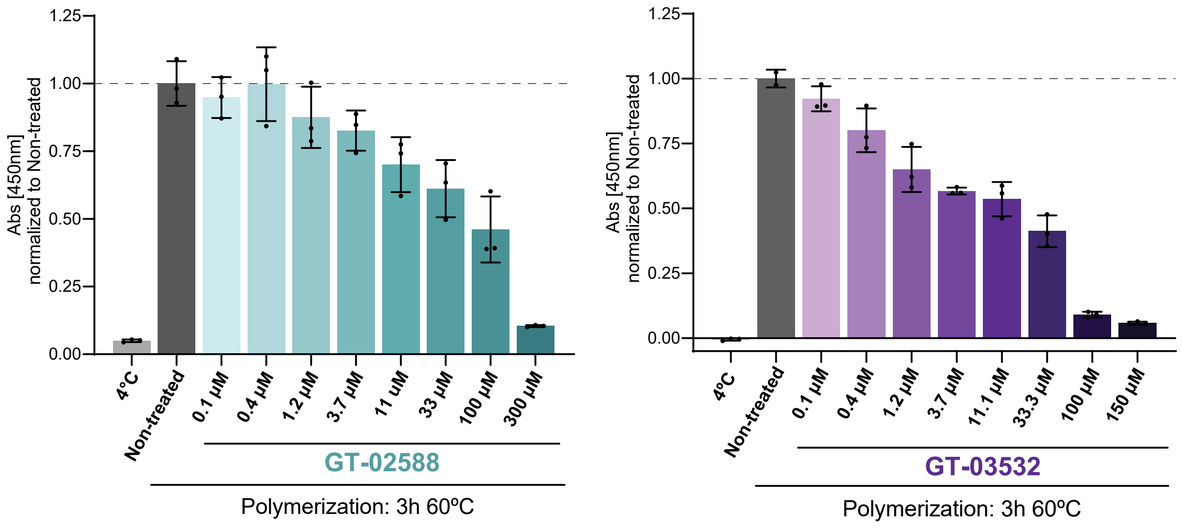
Applying our proprietary virtual screening methodology, a set of small molecules was selected and confirmed experimentally, and yielded a preferred hit series.

The hit series is the starting point for developing structurally targeted allosteric regulators (STARs) that allow correct folding of A1AT protein and restore its biological activity.



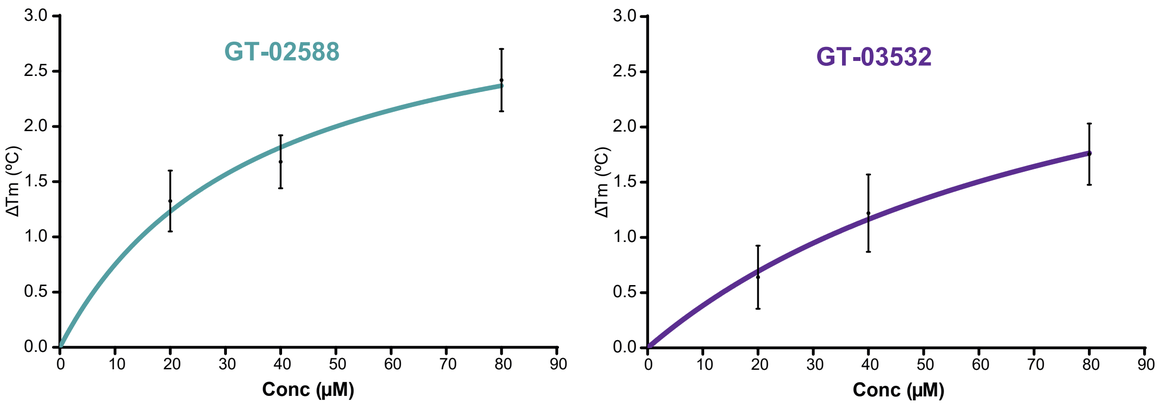
Aim: Prevent A1AT polymerization and restore its function using allosteric regulators

1 STARs inhibit induced WT human plasma-derived A1AT polymerization in a dose-response manner



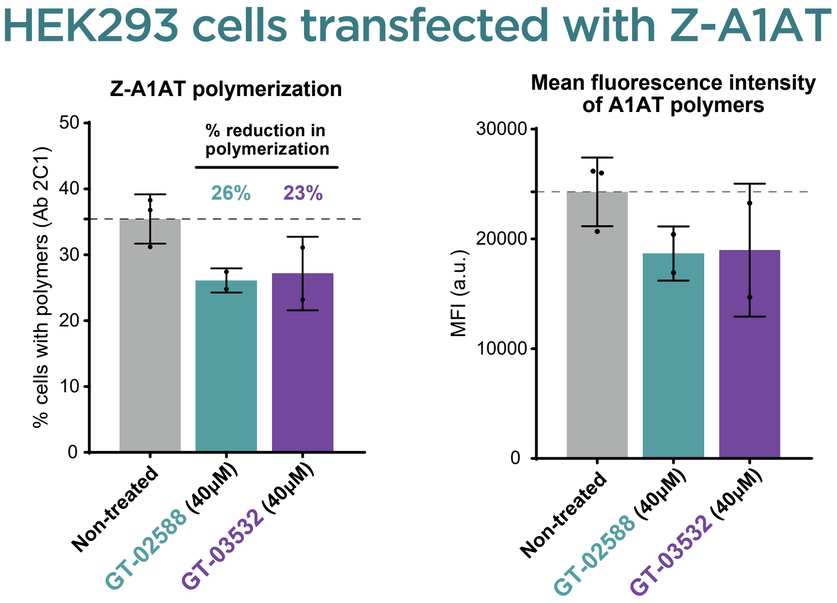
WT-A1AT protein purified from human plasma (0.1mg/mL) was incubated for 3h at 60°C to induce protein polymerization, in the presence or absence (non-treated) of GT compounds (GT-02588 and GT-03532) at different concentrations. ELISA for detection of polymerized A1AT was performed with 100ng/mL of protein using an anti-α1-antitrypsin antibody that detects all forms of A1AT as capture antibody, and 2C1 antibody to detect A1AT polymers as detection antibody to detect A1AT polymers. Results are presented as mean±SD.

2 DSF assay showed that STARs bind and stabilize WT A1AT



The thermal denaturation of WT-A1AT protein purified from human plasma (0.5µM) in the presence of GT compounds was monitored using the extrinsic fluorescent probe SYPRO™ Orange that binds to hydrophobic parts of proteins which are exposed as the protein unfolds. Unfolding curves were recorded from 20 to 85°C, at a scan rate of 1°C/min. Curves were smoothed, normalized, and analyzed using in-house software. The melting temperature (Tm) was calculated as the temperature at which half the protein is in the unfolded state. ΔTm is calculated as the value of Tm of the protein in the presence of compound subtracting the value of Tm in the absence of compound.

3 STARs reduce the formation of A1AT polymers in HEK293 cells transfected with Z-A1AT



HEK293 cells were transfected with Z-A1AT plasmid and cultured for 2 days at 37°C. GT compounds were added to the cell culture at the indicated concentrations and incubated for 48 hours. Cells were fixed, permeabilized and stained with LIVE/DEAD to determine the viability. Immunofluorescence staining was performed using 2C1 antibody to label polymerized forms of A1AT and a fluorescent secondary antibody. Samples were analyzed by flow cytometry (20000 cells/sample). Percentage of cells expressing A1AT polymers and the mean fluorescence intensity of the polymers were calculated. Results are presented as mean±SD.

Conclusions

SEE-Tx™ is a fast and cost-effective solution that has allowed us to identify structurally-targeted allosteric regulators (STARs) of the A1AT protein.

GT compounds showed prevention of polymerization in biochemical and cellular assays using WT or Z-A1AT protein.

Thermal stability assay confirmed that the selected compounds bind and stabilize the A1AT protein.

Gain Therapeutics has identified a novel chemical series that, by binding to A1AT restores its folding and could eventually improve its trafficking and activity, providing an opportunity for the treatment of A1AT deficiency.

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