DefiniGEN
DISEASE MODEL INNOVATION

Generating human preclinical data for candidate therapeutics against Alpha-1-antitrypsin deficiency

A1ATD is a disease which manifests in the liver as a result of an inherited mutation in the SERPINA1 gene (E342K ZZ being the most prevalent) and results in the misfolding of the A1AT protein, forming aggregates in the endoplasmic reticulum of the hepatocytes thereby causing liver damage. In addition, the lack of active A1AT protein in the bloodstream causes a failure to inhibit neutrophil elastase in the lungs, leading to chronic and life-threatening lung damage.

Background

A1ATD is a multifocal disease affecting the lungs and the liver. Efficacious treatments have been developed to alleviate disease progression in the lungs, but no equivalent treatments exist for the liver. The only curative option is liver transplant. Therefore, there is a clinical need to develop novel treatments for A1ATD-induced liver disease.

Currently there are several therapeutic techniques at various stages of clinical evaluation. These include modulation of A1AT polymer folding or transport through the targeting chaperone proteins and reducing A1AT polymer accumulation in hepatocytes via siRNA-mediated mRNA degradation or induction of autophagy-dependent protein polymer clearance.

As yet, no treatment has proven to be both safe and efficacious in the treatment of A1ATD-induced liver disease, meaning the clinical need remains unmet.

Through our unique and innovative differentiation platform, DefiniGEN have brought to market a unique service offering for the profiling and evaluation of therapeutic candidates being developed for Alpha-1-antitrypsin deficiency (A1ATD). Human hepatocyte-like cells are generated from iPSCs using a proprietary differentiation protocol and display relevant key hepatocyte markers. The Def-HEP A1ATD cells recapitulate what is seen in human A1ATD livers making it a phenotypically relevant model.

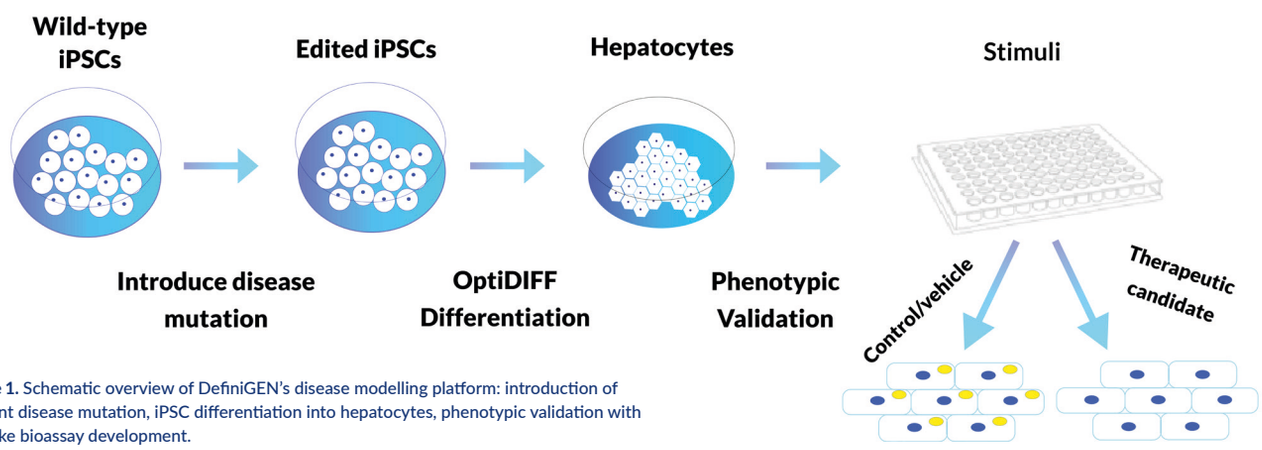
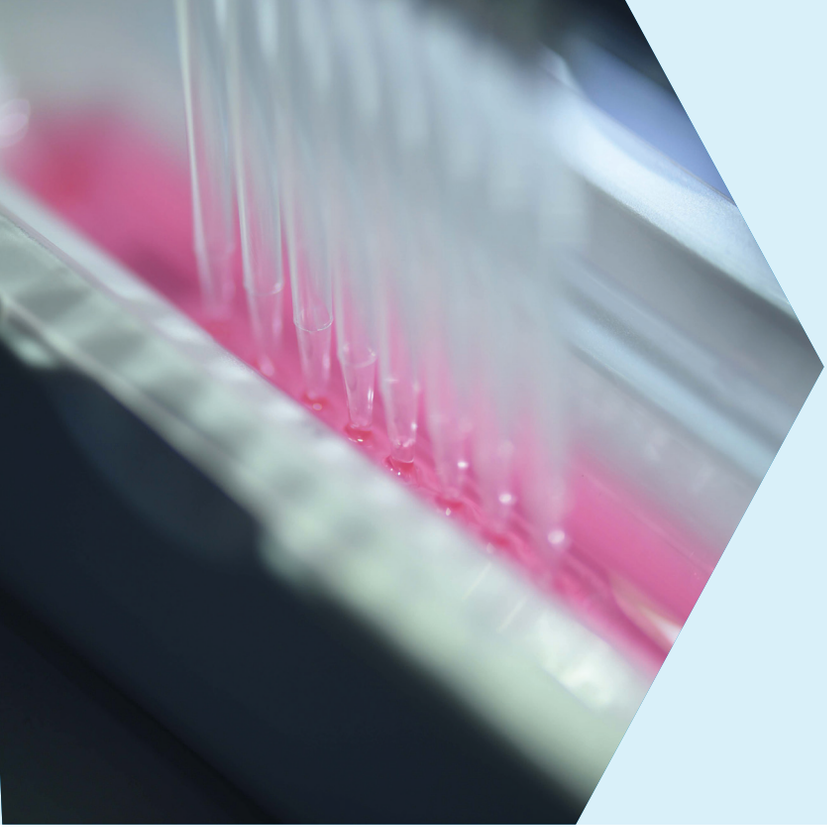


Figure 1. Schematic overview of DefiniGEN's disease modelling platform: introduction of relevant disease mutation, iPSC differentiation into hepatocytes, phenotypic validation with bespoke bioassay development.



Model development

Due to our proprietary differentiation protocol which derives a very pure Definitive Endoderm population early in the cell generation process, DefiniGEN's terminally differentiated hepatocytes have comparable levels of key markers (albumin, A1AT, HNF4a and AFP) to primary human hepatocytes (PHH). They display LDL incorporation, a demonstrably functional Urea cycle, gluconeogenesis / gylcogenolysis and have been characterised to show conservation of many cellular processes which are vital for in the *in-vitro* modelling of monogenic liver conditions.

DefiniGEN have an iPSC line which has been selected from many hundreds for its robust differentiation characteristics, and using CRISPR gene editing have generated a ZZ mutant with an otherwise healthy genetic background for primary screening activities. This model shows good reproducibility in polymeric A1AT expression as determined by IF across a 96 well plate, with CoV's typically sub 15%.

Assay development - polymer accumulation

Polymer build up in the livers of A1ATD patients, and DefiniGEN's model directly measures hepatic polymer accumulation which is also used as a clinical endpoint for the in-vivo testing of candidate therapeutics.

An optimised immunofluorescent bioassay for polymeric A1AT gives a reliable endpoint, and treatment windows for different molecular entities have been established.

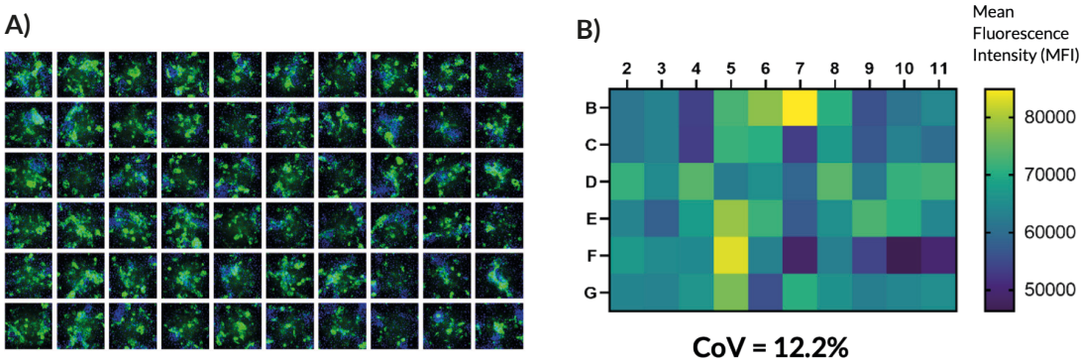


Figure 2. Intracellular accumulation of polymeric A1AT in Def-HEP A1ATD cells is uniform across the central wells of a 96 well plate . A) Immunostaining with polymer-specific A1AT antibody shows consistent levels of polymeric A1AT in cells. Representative images of 3 independent experiments. B) Quantification of the intracellular levels of polymeric A1AT in A1ATD Def-HEP across the central wells of a 96 wp (n=3).

siRNA based therapy

Def-HEP A1ATD cells are readily transducible or transfectable and lend themselves to the evaluation of gene therapy approaches, from siRNA to viral vectors or base-editing approaches. Our clients can send their compounds or reagents directly to our facility in Cambridge UK and data can typically be generated in 6-8 weeks.

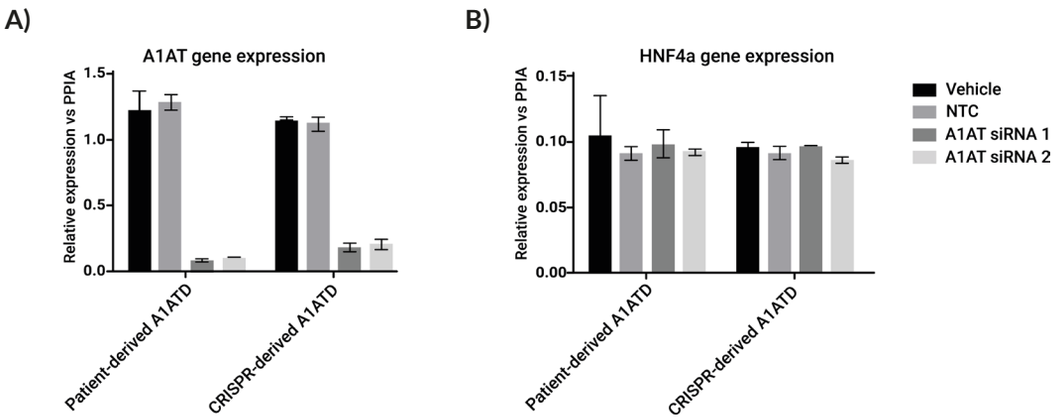


Figure 3. Gene expression of Def-HEP A1ATD 48h after transfection with scramble siRNA (NTC) and two different combinations of siRNA targeting SERPINA1 (A1AT). Gene expression of A1AT dramatically decreases with both sets of siRNAs (A) but other genes such as HNF4a (B) remain consistently expressed.

siRNA based therapy

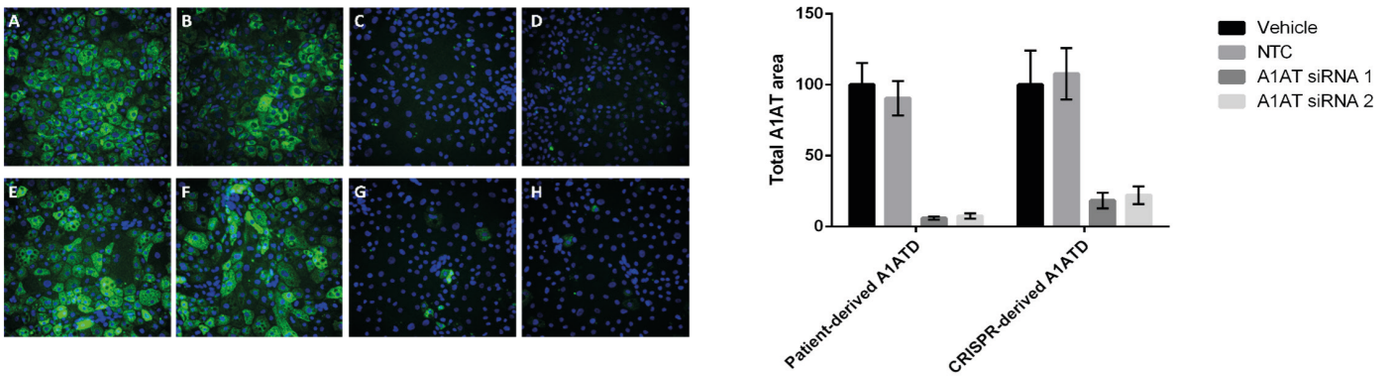


Figure 4. Immunofluorescence for polymeric A1AT. Representative pictures of polymeric A1AT staining for patient derived A1ATD hepatocytes transfected for 72h with Vehicle (A), Scrambled siRNA (B), and two different combinations of siRNA targeting SERPINA1 (C, D). Representative pictures of A1AT staining for CRISPR-derived A1ATD hepatocytes transfected for 72h with Vehicle (E), Scrambled siRNA (F), and two different combinations of siRNA targeting SERPINA1 (G, H). Quantification of polymeric inclusions of A1AT per cell in the different conditions.

Response to reference drug carbamazepine (CBZ)

Autophagy-inducing compounds including carbamazepine have been shown to accelerate the clearance of polymeric A1AT in hepatocytes, and DefiniGEN have used this compound as our standard positive control in every plate run for small molecule screens.

Recent advances in gene therapies and innovative small molecule approaches offer new hope for curative treatments, however there is a paucity of pre-clinical human models available to assess efficacy. DefiniGEN offer screening services on metabolically competent hepatocytes with a mature phenotype, to help support drug development for this indication and generate valuable human data for your candidate therapeutics. Our cells give robust responses to carbamazepine and other autophagy inducing small molecules.

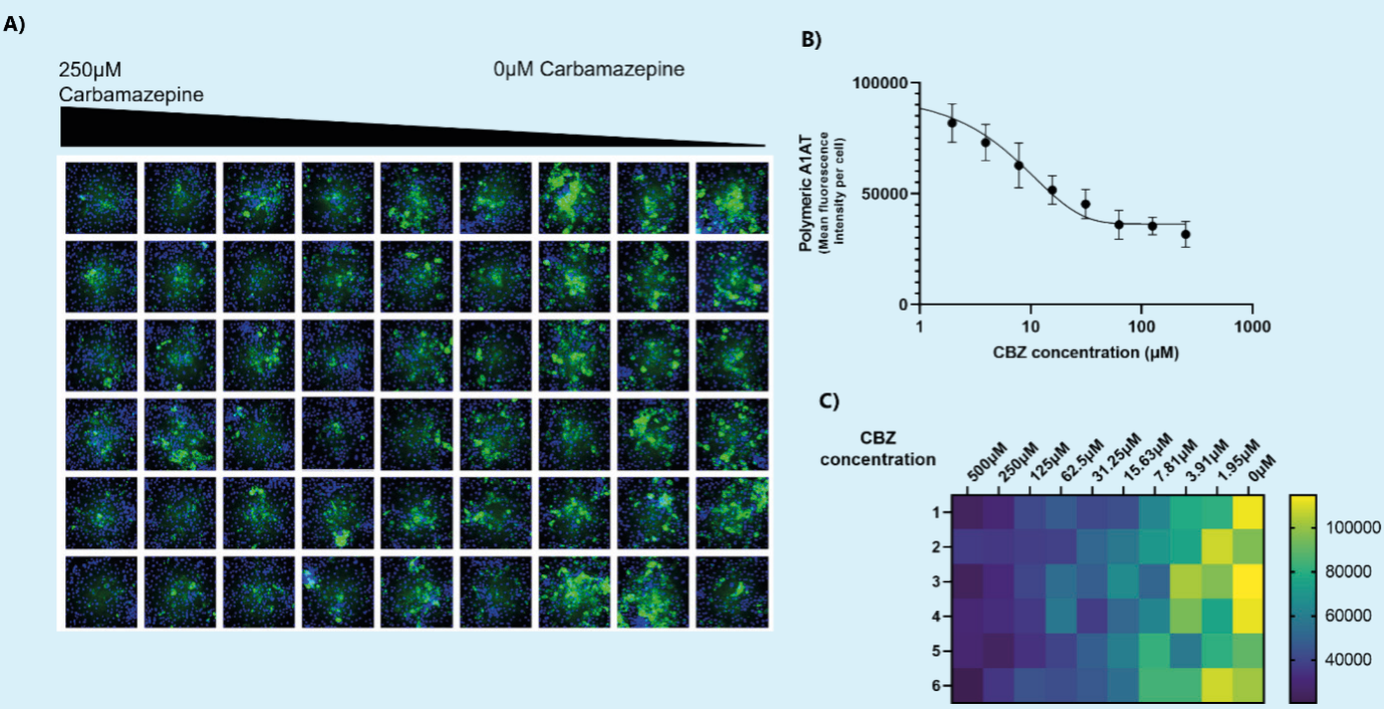


Figure 5. Intracellular accumulation of polymeric A1AT in A1ATD Def-HEP cells decreases in a concentration dependent-manner when treated with Carbamazepine (CBZ). A) Immunostaining with polymer-specific A1AT antibody shows decreased levels of polymeric A1AT in cells treated with CBZ in a concentration-dependent manner. B) Quantification of the intracellular levels of polymeric A1AT showing mean fluorescence intensity (MFI) per cell (n=4). C) Heatmap showing quantification of polymeric A1AT across the central wells of a 96 well plate.

Response to novel autophagy-inducing compounds

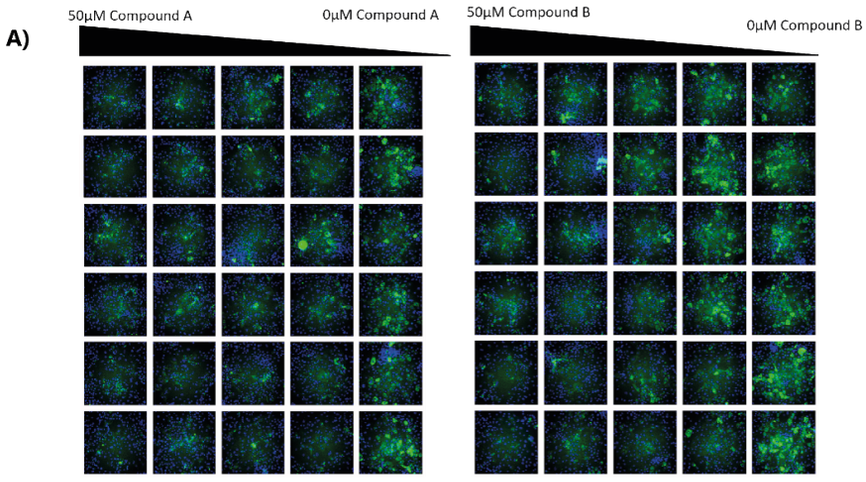


Figure 6. Intracellular accumulation of polymeric A1AT in Def-HEP A1ATD cells decreases in a concentration dependent-manner when treated with other compounds that increase autophagy. A) Immunostaining with polymer specific A1AT antibody shows decreased levels of polymeric A1AT in cells treated with two different compounds that induce autophagy. B) Quantification of the intracellular levels of polymeric A1AT in cells treated with different concentrations of compounds that induce autophagy

A1ATD patient-panel

To follow the initial primary screen in the CRISPR derived model, DefiniGEN also offer a panel of donor-derived hepatocytes generated from patients which suffered from the disease. This allows lead compound sets to be profiled across several donors, allowing some understanding of the clinical diversity of response which could be shown in the broader population. This panel includes male and female donors and different A1ATD genotypes.

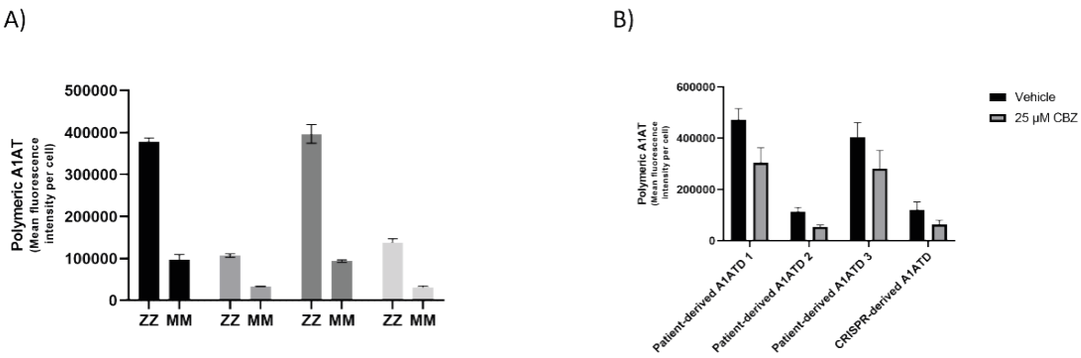


Figure 7. Intracellular accumulation of polymeric A1AT in Def-HEP A1ATD cells in a panel of 4 different donors. A) Immunostaining with polymer specific A1AT antibody shows increased polymer accumulation across a panel of different donors with the ZZ variant versus the isogenic MM variant. B) All diseased patients show a response to autophagy-inducing drug.

Below is some data showing treatment with a range of gene therapy approaches, from mRNA to plasmids, AAV to lentiviral constructs.

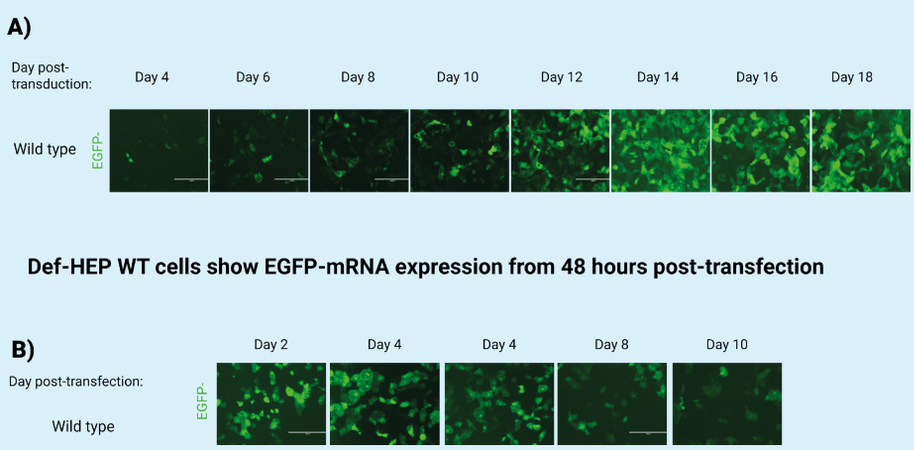
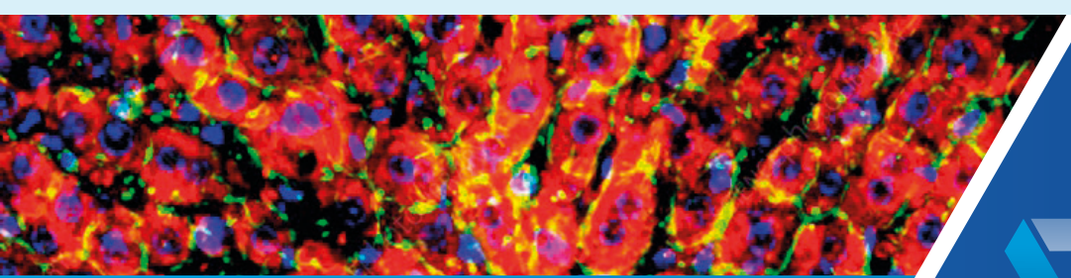


Figure 8. Immunofluorescence for EGFP. Representative pictures of EGFP expression in Def-HEP WT cells following AAV transduction [AAV3b vector] (A), and mRNA transfection [using lipofectamine Messenger Max] (B), in a time dependent manner.



If you would like to speak with an expert about your discovery program, please contact one of our experts at info@definigen.com

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