Proteomic analysis identified fazirsiran treatment-responsive protein biomarkers in patients with alpha-1 antitrypsin deficiency-associated liver disease

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Introduction

- Alpha-1 antitrypsin deficiency (AATD) is a rare genetic disease characterized by low levels of serum alpha-1 antitrypsin (AAT), which primarily affects the lungs and/or liver.[1]
- Patients with the protease inhibitor (Pi)*ZZ genotype express misfolded AAT (Z-AAT), resulting in hepatic Z-AAT aggregates and reduced antiprotease activity in the lungs.[1,2]
- Accumulation of proteotoxic Z-AAT polymers leads to hepatic cell stress, inflammation and cell damage, and liver fibrosis with increased extracellular matrix turnover.[3]
- Currently no pharmacological treatments for AATD-associated liver disease (AATD-LD) exist.[4]
  - Fazirsiran is an investigational small interfering RNA undergoing phase 3 development in patients with AATD-LD (NCT05677971).
- Proteomics data to identify biomarkers relevant for therapeutic targeting and to elucidate disease pathophysiology are limited.

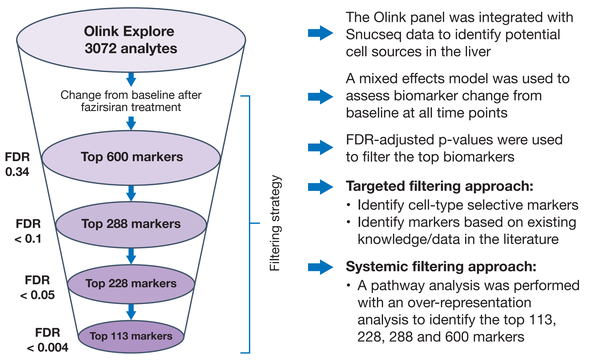
Objective

- To detect treatment-responsive protein biomarkers and cellular pathways by leveraging a novel proteomic platform for biomarker discovery using serum samples from patients with AATD-LD treated with fazirsiran.

Methods

- Olink Explore 3072, a high-throughput dual-antibody-based proteomics platform, was utilized for protein biomarker discovery.
- Serum samples were assessed at baseline and at 4, 16, 24, 28 and 48 weeks post-treatment initiation from 16 adults with AATD-LD, a Pi*ZZ genotype, and biopsy-proven liver fibrosis who participated in AROAAT-2002 (NCT03946449), a phase 2, open-label trial of fazirsiran (100 or 200 mg); treatment with fazirsiran resulted in > 80% reduction in serum and liver Z-AAT. [5]
  - Additional study design information has been previously described.[5]
- Olink data were integrated with single nucleus RNA-sequencing (Snucseq) data to map expressed proteins to potential source cells in the liver and improve data interpretability.
  - The Snucseq data consisted of samples from four patients with non-alcoholic steatohepatitis (NASH)/metabolic dysfunction-associated steatohepatitis (MASH) and two healthy controls.
- A mixed effects model was applied to measure biomarker change from baseline over time and a false discovery rate (FDR)-adjusted p-value was used to select the top biomarkers (**Figure 1**).
- Differentially expressed protein (DEP) and pathway analyses were also conducted.

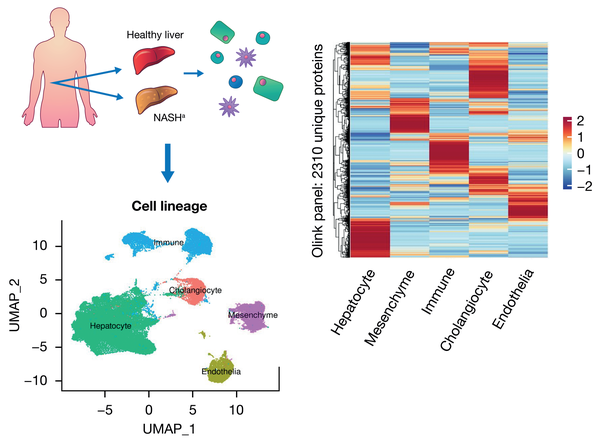
Figure 1. Statistical and bioinformatic approaches identified fazirsiran-responsive serum protein markers



Results

- Integration of the Olink panel with the Snucseq data is shown in **Figure 2**.
- Fazirsiran treatment resulted in continuous and sustained reductions in DEPs in serum through 48 weeks (**Figure 3A**).
- DEPs were primarily mapped to hepatocytes, mesenchymal cells, immune cells (T cells and macrophages), cholangiocytes and endothelial cells (**Figure 3B**).
  - DEPs revealed potential common mechanisms associated with liver fibrosis between AATD-LD and other liver diseases, including NASH/MASH and non-alcoholic fatty liver disease/metabolic dysfunction-associated fatty liver disease.
  - Of the liver mesenchymal cell-enriched proteins, many were components of the extracellular matrix associated with hepatic stellate cell activation and liver fibrosis (**Figure 4**).
  - Downregulated proteins that mapped to hepatocytes were associated with cell stress and apoptosis.

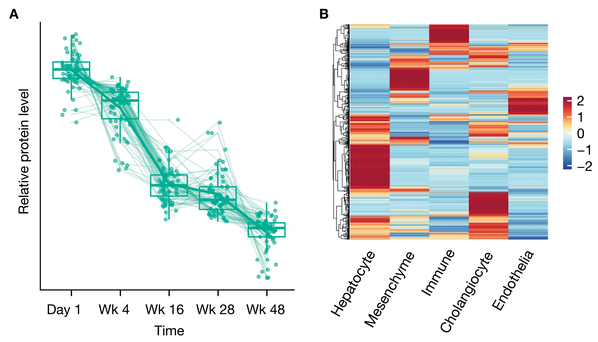
Figure 2. Integration of the Olink panel with Snucseq data suggested liver cell sources for the circulating biomarkers



Rows in the heatmap correspond to enrichment of transcripts coding for the protein markers included in the Olink panel in respective cell lineages and are scaled (z-score transformation indicating high [red] and low [blue] expression).

[a]At study conception, NASH was the accepted term for this disease. Following a multisociety Delphi consensus initiative, this term has been updated to MASH.[6]

Figure 3. (A)[a] Fazirsiran treatment led to continuous and sustained reductions of proteins in the serum; (B)[b] mapping DEPs to Snucseq data helped to define liver cell origin and contribution of distinct liver cell types to the observed serum DEPs

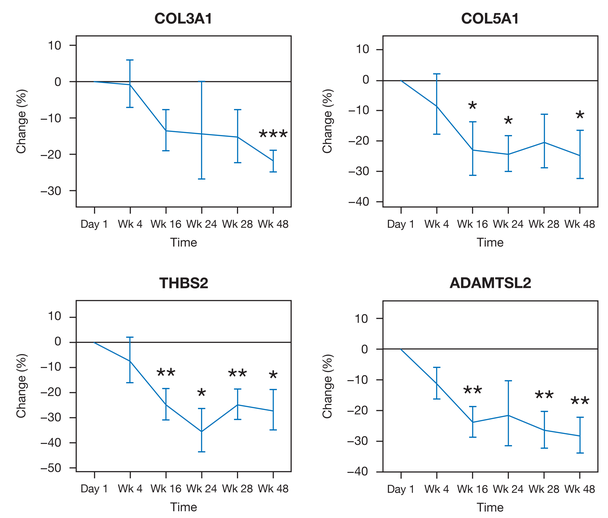


[a]Each circle represents a protein; the box plots shows the median (thick horizontal line), upper and lower interquartile range (thin horizontal lines) and the range (vertical line at each time point) of relative DEP abundance. Baseline samples were collected pre-dose on Day 1.

[b]Rows in the heatmap correspond to enrichment of fazirsiran treatment-responsive proteins in respective cell lineages and are scaled (z-score transformation indicating high [red] and low [blue] expression).

- Ingenuity pathway analysis identified molecular pathways that were improved by fazirsiran treatment (**Figure 5**).
  - Reduced levels of proteins linked to hepatocyte and cell death pathways, including p38 mitogen-activated protein kinase and immunogenic cell death signalling.
  - Decreases in proteins linked to inflammatory response pathways, including Th1/2 activation and cytokine expression; dendritic and natural killer cell crosstalk; macrophages, fibroblasts and endothelial cells; and rheumatoid arthritis-related markers.
  - Reduction in markers associated with liver fibrosis, including moderation of hepatic stellate cell activation, retinoids and wound healing.

Figure 4. Fazirsiran treatment led to significant reductions in circulating protein biomarkers mapped to mesenchymal cells and associated with liver fibrosis and damage



*p < 0.05, **p < 0.01, ***p < 0.001. The mean and standard error are plotted for each time point. Baseline samples were collected pre-dose on Day 1.

Figure 5. Ingenuity pathway analysis showed that fazirsiran-induced proteomic changes from baseline through 48 weeks were linked to the reduced activity of molecular pathways involved in hepatic cell stress and damage, apoptosis, inflammation, extracellular matrix turnover and liver fibrosis

Number of markers included in the pathway analysis: 288	600	
*		Dopamine receptor signalling
*		NAD biosynthesis II (from tryptophan)
**	*	Retinoate biosynthesis I
*		Methylglyoxal degradation III
**	*	Tryptophan degradation to 2-amino-3 carboxymuconate semialdehyde
**	*	Noradrenaline and adrenaline degradation
*		γ-glutamyl cycle
*		Catecholamine biosynthesis
		Glutathione redox reactions II
*	**	Acute phase response signalling
*	*	Neuroprotective role of THOP1 in Alzheimer's disease
	*	p38 MAPK signalling
	*	Immunogenic cell death signalling pathway
*	**	PI3K/AKT signalling
	*	Superpathway of methionine degradation
*	*	Tryptophan degradation X (mammalian, via tryptamine)
*	*	Xenobiotic metabolism PXR signalling pathway
**	**	Granulocyte adhesion and diapedesis
	*	IL-6 signalling
**	**	STAT3 signalling
	*	Retinol biosynthesis
	**	Agranulocyte adhesion and diapedesis
	*	Glucocorticoid receptor signalling
**	**	Role of macrophages, fibroblasts and endothelial cells in rheumatoid arthritis
**	**	LXR/RXR activation
	**	PPAR signalling
*	**	Role of osteoblasts, osteoclasts and chondrocytes in rheumatoid arthritis
	**	SPINK1 pancreatic cancer pathway
	**	IL-15 production
	*	PTEN signalling
	**	CLEAR signalling pathway
	**	Crosstalk between dendritic cells and natural killer cells
	**	Th1 and Th2 activation pathway
*	**	Role of chondrocytes in rheumatoid arthritis signalling pathway
	**	Cardiac hypertrophy signalling (enhanced)
**	**	Hepatic fibrosis/ hepatic stellate cell activation
	**	Osteoarthritis pathway
	*	Axonal guidance signalling
	**	Differential regulation of cytokine production in macrophages and T helper cells by IL-17A and IL-17F
	*	Leukotriene biosynthesis
**	**	LPS/IL-1 mediated inhibition of RXR function
**	**	Wound healing signalling pathway
	*	Apelin liver signalling pathway
**	**	Pathogen induced cytokine storm signalling pathway

*p < 0.001, **p < 0.0001.

Conclusions

- This study leveraged a novel proteomic platform for biomarker discovery in patients with AATD-LD, identified candidate biomarkers reflecting disease progression, and demonstrated potential benefit of fazirsiran treatment in reducing cellular stress and damage, apoptosis, inflammation and extracellular matrix turnover/fibrosis.
- Collectively, these findings provide molecular evidence to support potential clinical benefit of fazirsiran in patients with AATD-LD.
- Biomarkers identified in these analyses may have clinical utility but require validation in larger studies.
- The study was limited by:
  - the small sample size (n = 16)
  - the lack of comparator data from healthy patients or patients who received placebo; future research should generate additional Olink proteomic data from these comparator populations.
- Quantitative assays, such as enzyme-linked immunosorbent assays, would prove beneficial in validating findings from this study and bridging the gap to clinical practice.

Abbreviations

ADAMTSL2, disintegrin and metalloproteinase domain with thrombospondin motif-like protein 2; C7, complement component 7; CLEAR, coordinate lysosomal expression and regulation; COL3A1, collagen type III alpha 1 chain; COL5A1, collagen type V alpha 1 chain; DEP, differentially expressed protein; FABP1, fatty acid binding protein 1; FDR, false discovery rate; IL-6, interleukin 6; IL-15, interleukin 15; IL-17A, interleukin 17A; IL-17F, interleukin 17F; ITGBL1, integrin subunit beta like 1; KRT18, type I intermediate filament chain keratin 18; LPS/IL-1, lipopolysaccharide/interleukin 1; LXR/RXR, liver X receptor/retinoid X receptor; MAPK, mitogen-activated protein kinase; MASH, metabolic dysfunction-associated steatohepatitis; NAD, nicotinamide adenine dinucleotide; NASH, non-alcoholic steatohepatitis; PI3K/AKT, phosphoinositide-3-kinase/protein kinase B; PPAR, peroxisome proliferator-activated receptor; PTEN, phosphatase and tensin homolog; PXR, pregnane X receptor; Snucseq, single cell nucleus RNA-sequencing; SPINK1, serine protease inhibitor kazal type 1; STAT3, signal transducer and activator of transcription 3; Th1, type 1 T helper; Th2, type 2 T helper; THBS2, thrombospondin 2; THOP1, thimet oligopeptidase; UMAP, uniform manifold approximation and projection for dimension reduction; Wk, week.

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References

References can be found in the supplementary material accessed via the QR code.

Disclosures

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