Serum metabolomic, lipidomic and proteomic profiling identifies new biomarkers associated with the treatment of denifanstat, a fatty acid synthase inhibitor, in NASH patients

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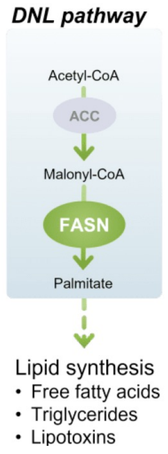
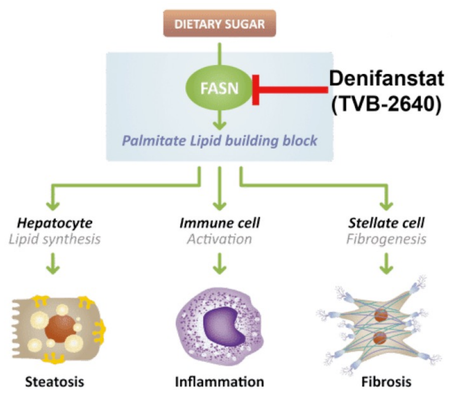
Introduction

- The *de novo* lipogenesis (DNL) pathway is elevated in NASH patients, and converts dietary sugars to palmitate, the building block for lipid synthesis.
- Fatty acid synthase (FASN) is the last committed step in DNL and therefore provides an approach to target three hallmarks of NASH; steatosis, inflammation and fibrosis (1, 2).
- The FASN inhibitor denifanstat (TVB-2640) is in Phase 2b development for NASH.
- The Phase 2a FASCINATE-1 study showed that denifanstat significantly reduced liver fat in NASH, and decreased disease biomarkers including ALT, CK-18, PRO-C3 and lipotoxins (3). This confirmed the expected mechanism of action.
- NASH is a complex and heterogeneous disease. Enrichment of patients most likely to respond to a specific therapy is a critical part of NASH drug development and has potential to improve response rates and direct patients to the most appropriate treatment.

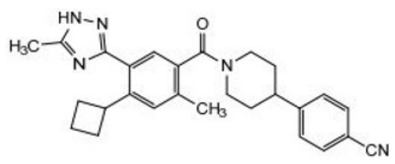
DNL pathway



FASN inhibition targets 3 key cell types



Denifanstat



Clinical development of denifanstat

Phase 1 Studies PK, PD	✓ Excellent PK profile, half life ~12 hr ✓ Once-daily, oral, tablet ✓ Proof of pharmacodynamic effect; inhibits hepatic de novo lipogenesis
Phase 2a NASH 12-week liver fat FASCINATE-1	✓ Proof of disease activity in NASH patients; consistent with expected MOA o Decreased liver fat o Decreased markers of inflammation o Decreased markers of fibrosis ✓ **Exploratory lipidomic screen for predictive markers of liver fat response**
Phase 2b NASH 52-week biopsy FASCINATE-2	✓ Ongoing ✓ Fully enrolled ✓ Interim analysis end of 2022, non-invasive tests

Aims

- To evaluate baseline lipidomic markers that predict liver fat response to denifanstat.
- To determine changes of serum proteins in response to denifanstat treatment

Methods

- Study design and results of the Phase 2a FASCINATE-1 study have been previously described (3).
- Baseline blood samples were profiled by LC/MS/MS for ~470 metabolites at OWL laboratories. Results from denifanstat 50 mg were analyzed by two different nonlinear regression machine learning algorithms (Random Forest and Support Vector Machine) to identify a biomarker panel that predicted liver fat response by MRI-PDFF. Samples were split into training and validation cohorts with 50x cross-validation using liver fat as a continuous variable
- Serum proteomic analysis (SomaScan assay) was performed by SomaLogic (Boulder, USA) for placebo and denifanstat 50mg-treated groups in the US cohort

References
(1) O'Farrell et al., 2022. Scientific Reports. doi.10.1038/s41598-022-19459-z
(2) Seyed et al., 2019. Hepatology. doi.org/10.1002/hep.31000
(3) Loomba et al., 2021.Gastroenterolog. doi:10.1053/j.gastro/2021.07.025
Acknowledgements
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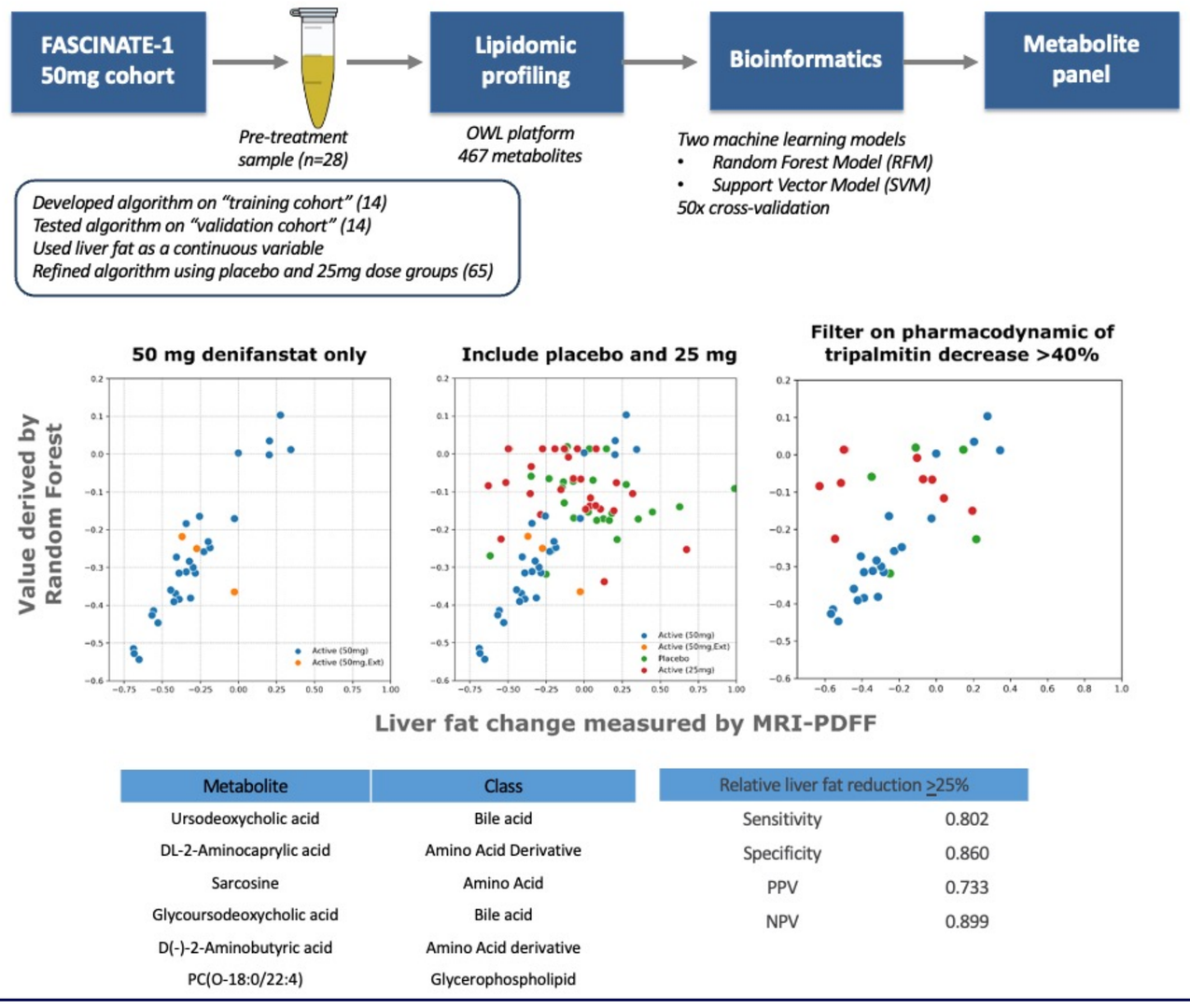
Results – predictive signature



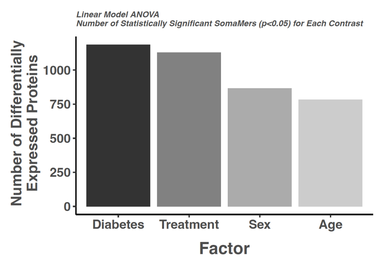
Metabolite	Class
Ursodeoxycholic acid	Bile acid
DL-2-Aminocaprylic acid	Amino Acid Derivative
Sarcosine	Amino Acid
Glycoursodeoxycholic acid	Bile acid
D(-)-2-Aminobutyric acid	Amino Acid derivative
PC(O-18:0/22:4)	Glycerophospholipid

Relative liver fat reduction ≥25%	
Sensitivity	0.802
Specificity	0.860
PPV	0.733
NPV	0.899

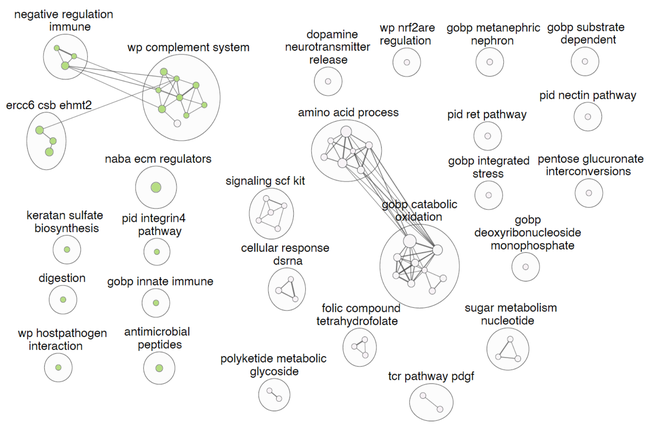
Results – serum proteomics

Enriched pathways are associated with denifanstat

Diabetes and denifanstat treatment are key factors to alter serum proteins



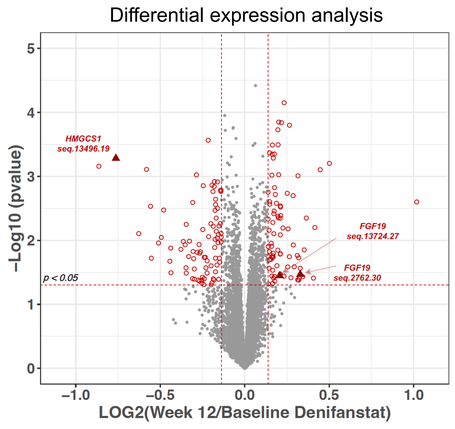
Pathway analysis of differential expressed proteins



Gray: pathways are down-regulated by denifanstat
Green: pathways are up-regulated by denifanstat

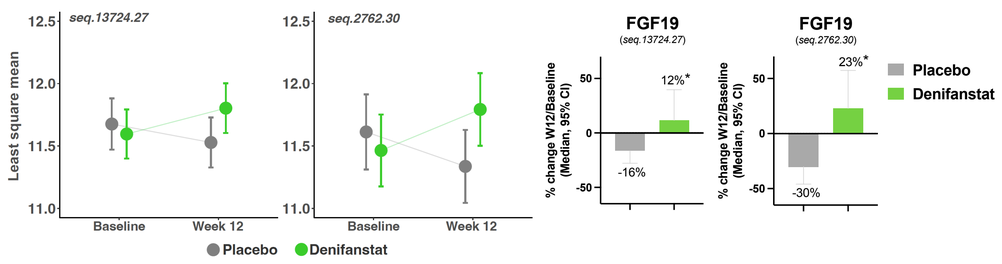
Serum proteins changed by denifanstat

Differential expression analysis



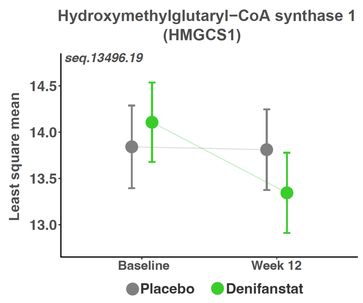
Denifanstat increased serum FGF19

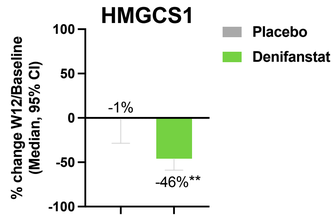
Fibroblast Growth Factor 19 (FGF19)



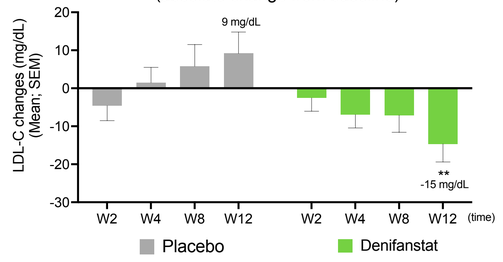
Denifanstat reduced serum HMGCS1 proteins and LDL-cholesterol

Hydroxymethylglutaryl−CoA synthase 1 (HMGCS1)





LDL-cholesterol (absolute change from baseline)



Conclusions

- A preliminary predictive lipidomic marker panel was identified that predicts liver fat response to denifanstat. This panel will be prospectively assessed to predict liver fat response in the Ph2b FASCINATE-2 trial, including week 26 interim expected 1H 2023, and subsequent week 52 liver biopsy results
- Serum proteomic analysis revealed that FGF19 was increased by denifanstat in NASH patients, suggesting that denifanstat may play a role in regulating bile acid synthesis, glucose and lipid metabolism through FGF19/FGFR4 signaling
- Serum HMGCS1 was decreased by denifanstat, concomitant with reduced circulating cholesterol and LDL-C, suggesting that denifanstat decreased HMGCS1 proteins in the liver, thereby reducing cholesterol synthesis
- FASCINATE-2 Ph2b biopsy study is ongoing with NASH patients receiving denifanstat 50mg QD; full interim analysis expected in 1H 2023
- Denifanstat has the potential to be a foundational treatment for NASH