The FASN inhibitor TVB-2640 is efficacious in a new 3D human liver microtissue model of NASH

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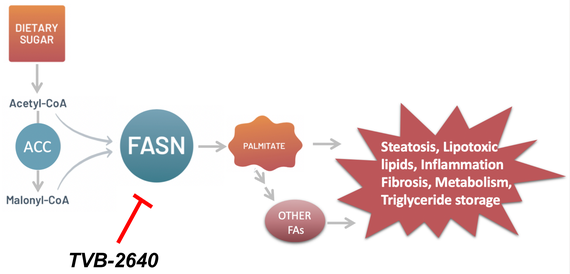
Introduction



FASN in NASH

- De novo lipogenesis (DNL) is upregulated in NASH and drives steatosis and lipotoxicity
- Fatty acid synthase (FASN) is the last committed step in the DNL pathway and produces palmitate
- Palmitate is not only a building block for lipids, but a signaling molecule that activates the inflammasome and TLR pathways, impacting inflammation and fibrosis in NASH

TVB-2640

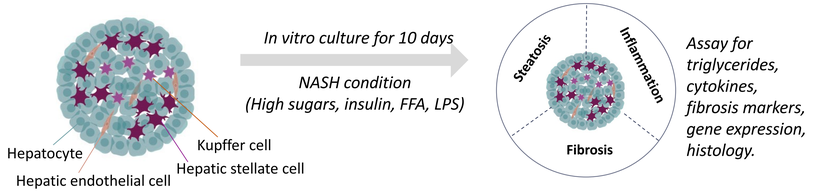
- Once-a-day oral small molecule
- Potent with FASN EC_{50} approx. 50 nM
- Excellent and predictable human PK profile
- Inhibits hepatic DNL up to 90% in human ^{13}C-acetate tracer study[1]
- First-in-class FASN inhibitor

TVB-2640 reduced liver fat in NASH patients in Ph2 FASCINATE-1 trial

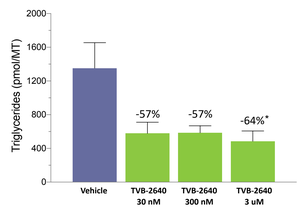
- 61% patients treated with 50 mg TVB-2640 had ⩾ 30% reduction in liver fat by MRI-PDFF, vs 11% in placebo
- Also decreased inflammation and fibrosis markers
- Oral presentation EASL ILC Abstract AS074, Loomba et al., 2020

Results in liver microtissue (LMT) model
TVB-2640 inhibits three hallmarks of NASH; steatosis, inflammation and fibrosis

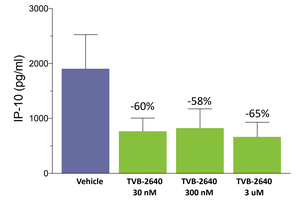
- **Primary human cell NASH model**
- Contains 4 liver cell types
- Mimics human NASH by culture with glucose, fructose, fatty acids, insulin, LPS.
- Developed and run by InSphero



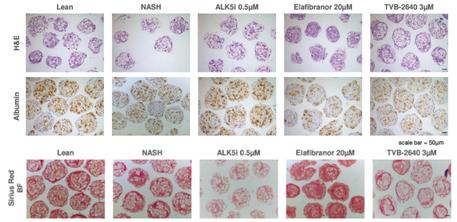
Steatosis Cellular TGs



Inflammation IP-10



Fibrosis Sirius Red

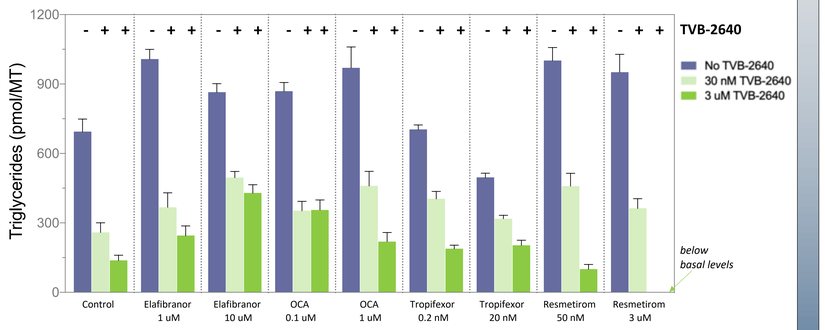


*LMTs were treated for 10 days with TVB-2640 in full NASH conditions (high sugars, FFA, insulin, LPS). Supernatants were tested for IP-10 levels on the Luminex platform (day 5). LMTs were lysed and assayed for TG levels using Glycerol/TG Glo kit (Promega) (day 10), corrected for basal TG levels. Results are mean +/-SEM of at least 4 LMT replicates per condition. *p<0.05 unpaired T test with Welch's correction. For histology, a minimum of 16 LMTs (day 10) were pooled, processed and stained as indicated, 20x objective. TG; triglycerides, IP-10; interferon-inducible protein-10.*

Combination Study Cellular TGs

Inhibitor	Class
Elafibranor (GFT505)	PPAR α/δ agonist
Obeticholic acid / OCA (INT747)	Bile acid analog FXR agonist
Resmetirom (MGL-3196)	THR-β agonist
Tropifexor (LNJ452)	Non bile acid FXR agonist

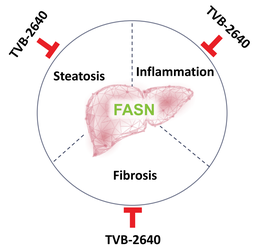
- Dose levels were selected based on published plasma levels in NASH clinical studies and/or in vitro cellular potency data
- No cytotoxicity was observed with the combinations based on LDH assays on day 3, 5, 7, 10
- Elafibranor, Obeticholic Acid, Resmetirom or Tropifexor did not show meaningful inhibition of cellular TGs in NASH conditions. Combination with TVB-2640 provided suppression of cellular TG similar to that observed with TVB-2640 alone.
- ALK5 inhibitor SB525334 and ASK1 inhibitor selonsertib were tested as single agents (not shown) and did not meaningfully suppress cellular triglyceride levels, but inhibited some inflammation and/or fibrosis parameters in this model
- Similar combination effect with TVB-2640 was observed in a second model (InSphero's DNL model) which lacks stellate cells and lacks exogenous FFA.



LMTs were treated for 10 days with TVB-2460 and/or other compounds in full NASH conditions (high sugars, FFA, insulin, LPS). LMTs were lysed and assayed for TG levels by ELISA. The first purple bar is NASH conditions with vehicle only. Results are mean+/-SEM of at least 6 LMT replicates per condition, corrected for basal TG levels.

Conclusions

- TVB-2640 reduced markers of steatosis (cellular TGs), inflammation (IP-10) and fibrosis (Sirius Red) in the human primary cell LMT model.
- Consistent with FASN inhibitor results in mouse NASH DIO and CCl4 models, previously published.
- Decreased cellular TGs by TVB-2640 in the LMT model recapitulates reduced liver fat by TVB-2640 in the recent Ph2 FASCINATE-1 study in NASH patients.
- Other NASH agents tested (Elafibranor, Obeticholic Acid, Resmetirom or Tropifexor) did not decrease cellular TGs in the LMT full NASH model as single agents, although decreases liver fat or steatosis have been shown in the clinic. These agents do not directly inhibit DNL, while ACC inhibitor GS-0976 had similar effect to TVB-2640 (not shown), consistent with direct DNL inhibition. The LMT full NASH model may not recapitulate the required mechanisms of altered lipid metabolism for other classes, and/or higher concentrations may be required.
- Addition of TVB-2640 to the other NASH agents tested provided suppression of cellular TG in the LMT model in full NASH conditions.
- Lipidomic profiling of supernatants by One Way Liver and assessment of fibrosis readouts such as ProC3 are ongoing.
- The InSphero LMT model provides a useful tractable and non-invasive human model in NASH.



Acknowledgements

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[1]*Syed-Abdul et al. 2020, Hepatology 72: 103.*

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