Combination of an Acetyl-CoA Carboxylase Inhibitor and Fibroblast Growth Factor-19 Reduced Tissue Triglyceride Content and Fibrosis in a 3D Human Liver Microtissue Model of Nonalcoholic Steatohepatitis

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Introduction

- Inhibition of the acetyl-coenzyme A carboxylase (ACC) isoforms ACC1 and ACC2 results in inhibition of fatty-acid (FA) synthesis and stimulation of FA oxidation, which may reduce lipotoxicity in nonalcoholic steatohepatitis (NASH)[1-4]
  - ACC inhibition also inhibits activation of hepatic stellate cells[5]
- Fibroblast growth factor-19 (FGF19) is secreted from the ileum in response to bile-acid absorption; in the liver, FGF19 signals through FGF receptor 4 to reduce bile-acid synthesis, and regulate glucose and lipid metabolism[6]
- In patients with advanced fibrosis (F3–F4) due to NASH, treatment with the ACC1/2 inhibitor (ACCi) firsocostat (FIR) alone led to significant improvements in liver steatosis; however, greater histologic improvements in fibrosis were observed only with the combination of FIR and the intestinally restricted farnesoid X receptor agonist cilofexor (CILO) compared with placebo or either monotherapy (NCT03449446)[7]

Objective

- To evaluate the effects of an ACCi and FGF19, alone and in combination, on lipid and fibrosis-related endpoints in a 3D human liver microtissue (MT) model of NASH

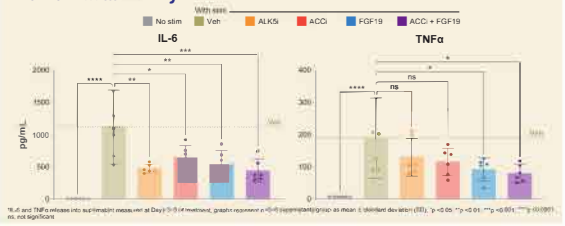
Methods

Study Design

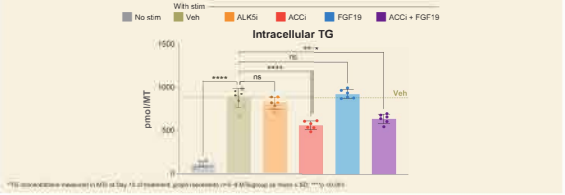
- Human liver spheroid MTs were formed using a scaffold-free coculture of primary human hepatocytes (IPHH_18), Kupffer and liver endothelial cells (IPHN_15), and hepatic stellate cells (IPHS_20)[8]
  - NASH features were induced in MT over 10 d with a proprietary mix of FFAs in media containing high concentrations of glucose, fructose, and insulin; lipopolysaccharide was administered once at Day 3
  - MT culture media, including FFAs and compounds, were collected and changed at Days 3, 5, 7, and 10
- The ACCi (an analog of FIR; 0.5 µM) or recombinant human FGF19 protein (500 ng/mL [PeproTech, Inc., Cranbury, NJ (Cat # 100-32)]) dissolved in phosphate-buffered saline without calcium/magnesium ions), or the combination, were administered at Days 0, 3, 5, and 7 for a total of 10 d; an ALK5i (0.5 µM [Selleck Chemicals, Houston, TX (Cat.# S1476)]) that blocks transforming growth factor-β signaling was used as a positive control
- IL-6 and TNFα concentrations in supernatants were measured using Human XL Cytokine Luminex® Performance Base Kit (R&D Systems, Inc., Minneapolis, MN)
- Total TG content in MTs was measured with Triglyceride-Glo™ Assay (Promega Corporation, Madison, WI); MTs were lysed and glycerol was released from TG by treatment with lipase before luminescence assay measuring total glycerol, and then amount of glycerol/MT (pmol/MT) was calculated
- Procollagen type I concentrations in supernatants were measured with the human procollagen type 1 kit (Cisbio Bioassays SAS, Codolet, France); procollagen type III concentrations in supernatants were measured with the procollagen-III-peptide (PIIINP) ELISA kit (Cisbio); concentrations of procollagen I and III were normalized to incubation times (2 or 3 d)
- SR staining[9]:
  - 18–20 MTs/condition were pooled and fixed with 4% paraformaldehyde for 1 h at room temperature; MTs were embedded in 1.7% agarose
  - SR staining was performed by Sophistolab AG (Muttenz, Switzerland); stained sections were imaged with a DMi8 inverted microscope (Leica Microsystems Inc., Buffalo Grove, IL) using a 20x objective
  - SR images were analyzed by FibroNest software (a digital pathology, quantitative, image analysis platform developed by PHARMANEST [Princeton, NJ]); only MT sections where ≥60% of the total area was within the image were analyzed with FibroNest
- Statistical significance was determined by 1-way analysis of variance, with repeated measures (IL-6, TNF-α, TG, and procollagen I and III) compared with Veh group

Results

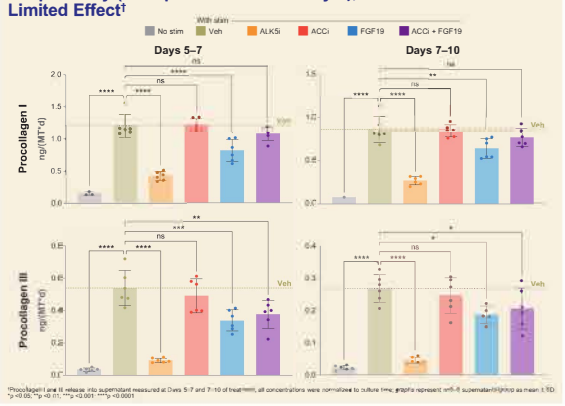
Combination of ACCi and FGF19 Reduced Secretion of IL-6 and TNFα From MTs: Days 3–5†



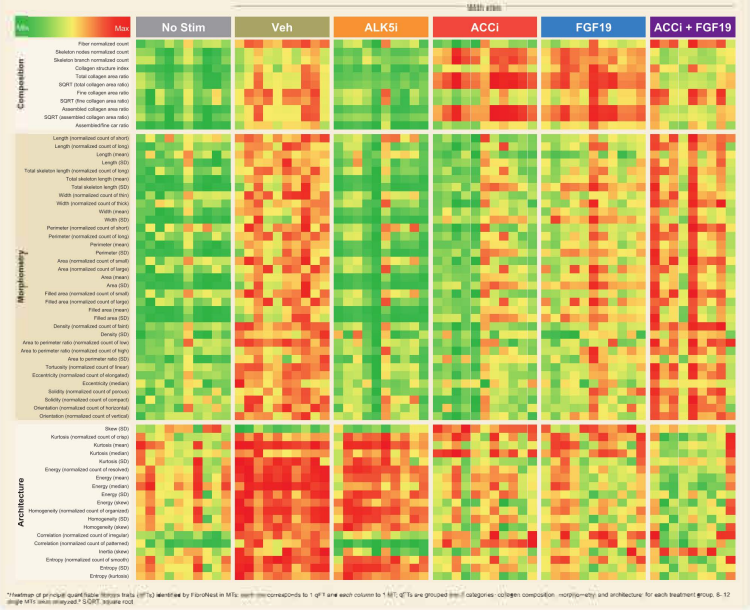
ACCi, But Not FGF19, Reduced TG by 36% in MTs at Day 10*



FGF19 Reduced Secretion of Procollagen I and III by 33% and 37%, Respectively (both p <0.001 vs veh: Day 7), But ACCi Alone Had Limited Effect†



While ACCi or FGF19 Alone Elicited Modest Improvements, the ACCi and FGF19 Combination Showed Further Reductions of Collagen Composition and Architecture Scores*



Conclusions

- Human liver MTs exhibited increased tissue TG, cytokine and procollagen secretion, and scores of principal qFTs after 10 d in NASH-inducing culture
- The combination of an ACCi and FGF19 reduced cytokine secretion and TG accumulation, and inhibited fibrogenesis in human liver MTs
- These data are similar to improvements in steatosis and fibrosis observed in patients with NASH treated with FIR and CILO combination therapy,[7] and support the utility of an ex vivo human model to evaluate NASH therapies

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