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Combinations of an Acetyl Coenzyme-A Carboxylase Inhibitor, FXR Agonist, and GLP-1R Agonist Inhibit Fibrosis Progression in the Rat Choline-Deficient, L-Amino-Acid–Defined, High-Fat Diet Model of Advanced Fibrosis

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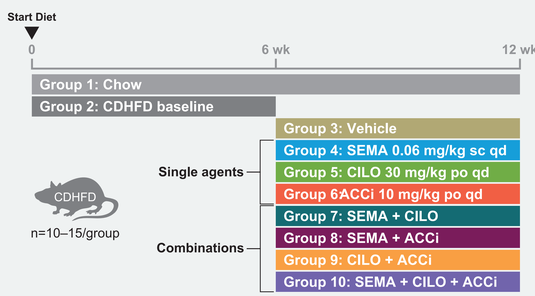
Introduction

- The combination of semaglutide (SEMA; glucagon-like peptide-1 receptor [GLP-1R] agonist), cilofexor (CILO; farnesoid X receptor [FXR] agonist) and firsocostat (FIR; acetyl coenzyme-A carboxylase inhibitor [ACCi]) is being investigated in patients with advanced fibrosis due to nonalcoholic steatohepatitis (NASH)
- SEMA is a GLP-1R agonist with many beneficial metabolic effects, such as improving glycemic control, increasing insulin sensitivity, and promoting weight loss; this reduces the influx of lipotoxic precursors, such as nonesterified fatty acids, from the periphery into the liver
  - In a 72-wk Phase 2 trial in patients with NASH and fibrosis stages 1–3, SEMA promoted NASH resolution, with the highest response rate observed in the SEMA 0.4 mg group (57% vs 20% in the placebo group)[1]
- CILO is an intestinally active FXR agonist that induces plasma fibroblast growth factor-19 levels, which reduces hepatic bile acid production, resulting in suppression of gluconeogenesis and de novo lipogenesis, thereby improving hepatocyte health; FIR is a liver-targeted ACC-1/2 inhibitor that blocks de novo lipogenesis and increases fatty acid oxidation, thereby improving hepatic lipotoxicity
  - In a Phase 2 trial in patients with advanced fibrosis (F3–4) due to NASH, treatment with CILO + FIR led to higher rates of fibrosis improvement (21%) vs placebo (11%) or either monotherapy (CILO: 12%, FIR: 12%) at Week 48[2]
- SEMA, CILO, and FIR target complementary mechanisms involved in the pathogenesis of NASH, and the combination of these agents is hypothesized to provide greater histologic benefits, including fibrosis improvement and NASH resolution, than possible with a monotherapy approach
- Furthermore, triple-combination SEMA + CILO + FIR therapy led to greater reductions in non-invasive assessments of liver steatosis and stiffness vs SEMA monotherapy over 24 wk in a Phase 2a study in patients with NASH[3]
- In the amylin liver NASH diet-induced mouse model, combinations of SEMA, CILO, and a FIR analog (GS-834356 [ACCi]) lowered liver fat, expression of fibrogenesis markers, and nonalcoholic fatty liver disease activity score more than monotherapies[4]; however, the mouse model developed mild–moderate fibrosis and may have limited utility in understanding the antifibrotic effects of the combinations

Objective

- To evaluate the effect of pairwise and triple combinations of SEMA, CILO, and ACCi on fibrosis progression in the rat choline-deficient, L-amino-acid–defined, high-fat diet (CDHFD) model of advanced fibrosis

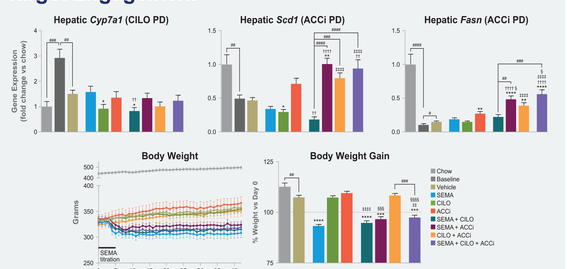
Methods



- Male Sprague Dawley rats were fed CDHFD (A16092003 [Research Diets, Inc., New Brunswick, New Jersey, USA]) for 12 wk, and dosed once daily with test articles (SEMA, CILO [GS-9674], and ACCi [GS-834356]) from Weeks 6 to 12 as indicated in the schematic; the SEMA dose was titrated over 5 d to prevent dramatic weight loss
- Separate groups of animals were necropsied after 6 wk of CDHFD feeding (baseline) or were fed with standard chow diet (Altromin 1324 [Altromin Spezialfutter GmbH & Co. KG, Lage, Germany]) for the duration of the study
- After 6 wk of dosing, animals were sacrificed in the ad lib fed state 24 h postdose, and plasma and tissues were processed for various endpoints
- Fibrosis was assessed in liver sections by quantifying the Picrosirius red (PSR)–positive area that stained collagen fibrils or by determining the hydroxyproline content biochemically in liver tissue
- Quantitative immunohistochemistry (IHC) was performed for collagen 1 α-1 (Col1a1; marker for fibrogenesis), α-smooth muscle actin (αSMA; marker for activated stellate cells), galectin-3 (marker of activated macrophages and Kupffer cells), and cluster of differentiation-68 (CD68; macrophage marker), and the images were analyzed for % positive area using Visiopharm software
- Gene expression was evaluated by reverse-transcription quantitative real-time polymerase chain reaction; data were normalized to housekeeping genes and expressed as fold change compared with vehicle
- Plasma levels of non-invasive biomarkers of fibrosis were determined by enzyme-linked immunosorbent assay
- % reductions in endpoints with test article treatment were normalized to vehicle (set to 0% reduction) and baseline/chow (set to 100% reduction) levels; heat map represents mean normalized reductions in endpoints
- Data are mean ± standard of the mean; statistical analysis was performed using Prism 8.3.1 (GraphPad Software, San Diego, California, USA; nonparametric 2-tailed Wilcoxon-Mann-Whitney test for comparison of 2 groups and Kruskal-Wallis test with Dunn's correction for multiple comparisons for comparison of >2 groups)
- #p ≤0.05, ##p ≤0.01, ###p ≤0.001, ####p ≤0.0001 as indicated; *p ≤0.05, **p ≤0.01, ***p ≤0.001, ****p ≤0.0001 vs vehicle; †p ≤0.05, ††p ≤0.01, †††p ≤0.001, ††††p ≤0.0001 vs SEMA monotherapy; ‡p ≤0.05, ‡‡p ≤0.01, ‡‡‡p ≤0.001, ‡‡‡‡p ≤0.0001 vs CILO monotherapy; §p ≤0.05, §§p ≤0.01, §§§p ≤0.001, §§§§p ≤0.0001 vs ACCi monotherapy; ¶p ≤0.05 vs SEMA + CILO + ACCi

Results

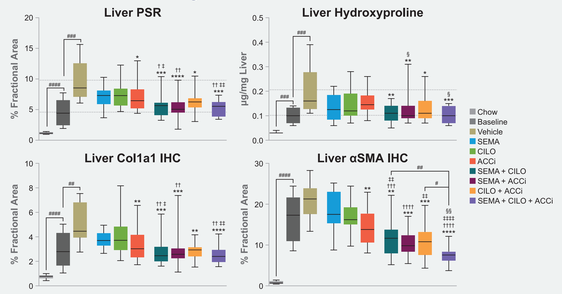
Target Engagement‖

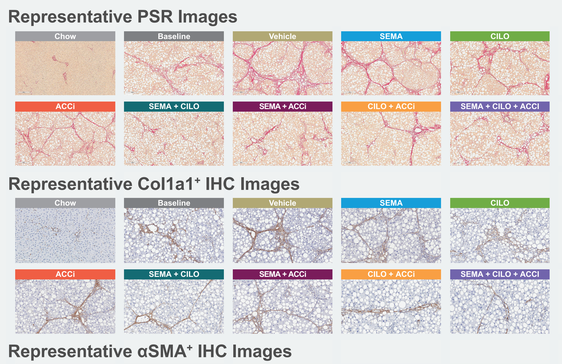


‖Hepatic gene expression to assess CILO (*Cyp7a1*) and ACCi (*Scd1* and *Fasn*) target engagement measured at end of study; longitudinal body weight measured over course of test article dosing and terminal weight gain. PD, pharmacodynamics.

- Expected target engagement was observed across all groups (reduction in body weight with SEMA, reduction in hepatic *Cyp7a1* expression with CILO, and increased *Scd1* and *Fasn* expression with ACCi)

Antifibrotic Efficacy‖





‖Liver fibrosis assessed by histologic PSR area quantitation and biochemical hydroxyproline content determination; fibrogenesis assessed by immunostaining for Col1a1+ and αSMA+ areas.

- Combinations of SEMA, CILO, and ACCi almost completely halted fibrosis progression in the rat CDHFD model, whereas monotherapies only had a partial effect on this parameter
- Reductions in Col1a1+ area were similar to the trend observed in PSR area
- Triple combination had greater effects on reducing αSMA+ area than the SEMA + CILO and CILO + ACCi pairwise combinations

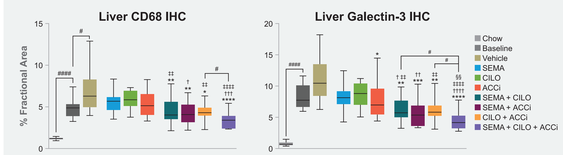
Noninvasive Biomarkers of Fibrosis‖

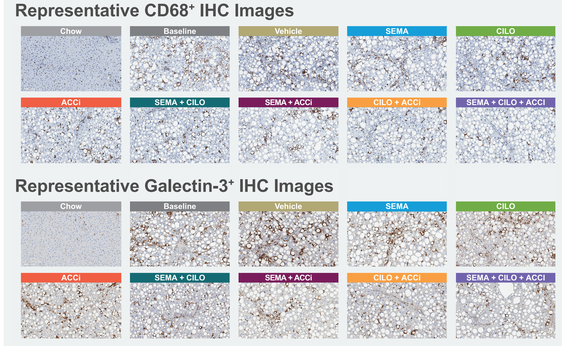
Parameter	Chow	Baseline	Vehicle	SEMA	CILO	ACCi	SEMA + CILO	SEMA + ACCi	CILO + ACCi	SEMA + CILO + ACCi
ALT (2 wk), U/L	49 ± 5[#]	287 ± 31	205 ± 22[#]	165 ± 16	160 ± 13	217 ± 19	137 ± 14[*]	174 ± 14[§]	156 ± 12[§]	119 ± 13[¶§]
AST (2 wk), U/L	83 ± 15[#]	390 ± 29	384 ± 41	208 ± 12[*]	288 ± 17	357 ± 40	183 ± 13[*†]	186 ± 10[*§]	290 ± 16[§]	175 ± 9[*¶§]
HA, ng/mL	41 ± 5[#]	76 ± 12	102 ± 15	79 ± 10	78 ± 12	63 ± 8	66 ± 5[§]	57 ± 6	47 ± 4[*†]	44 ± 6[*†§]
TIMP1, ng/mL	8 ± 0[#]	89 ± 8	89 ± 5	55 ± 5[*]	68 ± 4	70 ± 7[*]	47 ± 5[*†§]	38 ± 3[*†§]	54 ± 6[§]	29 ± 2[*†§§]
PIIINP, ng/mL	2 ± 0[#]	7 ± 1	11 ± 1	7 ± 1	7 ± 1	9 ± 1	6 ± 1[*]	5 ± 1[*]	6 ± 1[*]	4 ± 0[*†§]
A2M, µg/mL	18 ± 1[#]	221 ± 39	385 ± 53	171 ± 40[*]	264 ± 148	219 ± 41[*]	107 ± 20[*†]	103 ± 14[*§]	164 ± 19[§]	84 ± 10[*§§]
CK18-M30, mIU/mL	91 ± 17[#]	568 ± 38[#]	409 ± 43	223 ± 26[*]	310 ± 45	288 ± 34	197 ± 23[*§]	180 ± 21[*]	242 ± 33[§]	109 ± 15[*†§§]

‖Enhanced Liver Fibrosis test (ELF™; Siemens Healthcare GmbH, Erlangen, Germany) components: alanine aminotransferase (ALT), aspartate aminotransferase (AST), hyaluronic acid (HA), tissue inhibitor of metalloproteinase-1 (TIMP1), and type III procollagen (PIIINP); apart from ALT and AST, all other biomarkers represent terminal levels. α2M, α-2-macroglobulin; CK18-M30, cytokeratin 18 M30 fragment.

- Combinations were more efficacious than monotherapies to reduce circulating levels of plasma biomarkers of fibrosis, with the triple combination resulting in numerically greater reductions than the pairwise combination groups

Effects on Inflammation‖

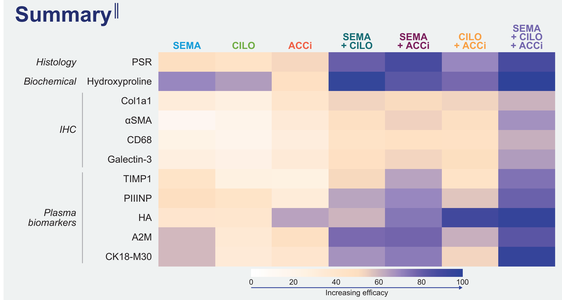




‖Hepatic inflammation assessed by immunostaining for CD68+ and galectin-3+ areas.

- Similar to other endpoints, combinations had greater effects on immune-cell markers than monotherapies, with the triple combination resulting in numerically greater reductions than the pairwise combinations

Summary‖



‖Data are % reduction in endpoints normalized to vehicle (0%) and baseline/chow (100%).

Conclusions

- Combinations of SEMA, CILO, and ACCi halted fibrosis progression in the rat CDHFD model of NASH and fibrosis
  - The totality of data suggest that the triple (SEMA + CILO + ACCi) combination has a greater effect on reducing multiple endpoints than the pairwise combinations
- These data support the ongoing clinical evaluation of the potential benefits of SEMA in combination with CILO and FIR in patients with advanced fibrosis due to NASH

References: 1. Newsome PN, et al. N Engl J Med 2021;384:1113-24; **2.** Loomba R, et al. J Hepatol 2020;73(suppl 1):S116-7 [abstr LBO04]; **3.** Alkhouri N, et al. AASLD 2020, abstr LO2; **4.** Norlin J, et al. Diabetes 2020;69(suppl 1):1810-P. **Acknowledgment:** This study was funded by Gilead Sciences, Inc. and Novo Nordisk A/S.